

4 February 1982

Test-tube babies pilloried again

Physicians' organizations in the United Kingdom are busying themselves with an examination of the ethics of in vitro fertilization and related developments. Why now? And to what end?

The British medical establishment seems bent on making an unpalatable mess of its consideration of the ethical problems occasioned by recent developments in the treatment of human infertility. This week, the ethical committee of the British Medical Association will have held a private but much publicized meeting to brood about the ethical implications of *in vitro* fertilization and the possibility of setting up banks of frozen sperms, oocytes and even embryos. And the Royal College of Obstetricians and Gynaecologists is embarking on a joint exercise with the same objectives. Naturally enough, an army of pundits has joined in the fuss, announcing to an astonished world that a new revolution of technique has suddenly taken place and that a novel set of legal conundrums has been created overnight. Whether or not physicians are enlightened by the work of the committees now hard at work, it is certain that the public will be confused.

The issues provoked by human embryology are by no means novel. For the past fifteen years, Mr Patrick Steptoe and Dr R.G. Edwards have made no secret of their ambitions to use *in vitro* fertilization of oocytes as a means of treating human infertility. The novelty is merely that their techniques are now reproducibly successful, in Britain but also in other places (see R.G. Edwards, *Nature* 293, 253; 1981). Although first thought of as a technique for treating female infertility, *in vitro* fertilization has also turned out to be a means of arranging for the conception of a live fetus by an oligospermic male partner to a marriage.

Strictly speaking, however, these novel developments occasion no novel ethical or legal problems. So much was made clear in the report in 1974 of a committee of the British Association for the Advancement of Science (see *Our future inheritance: Choice or chance* by Walter Bodmer and Alun Jones, OUP). For it seems generally agreed that conception is preferable to childlessness within a marriage, and that *in vitro* fertilization using a husband's sperm is preferable to fertilization (by artificial insemination) with male gametes from some sperm bank or donor. It may be appropriate (but can hardly be necessary) that physicians should have guidance in advising their patients when one form of treatment or the other may be advisable; they are more likely themselves to be guided by specialists in the field. It is entirely inappropriate that they should be given the false impression that entirely novel issues have been raised.

One particular red herring, wrongly linked with the practice of

in vitro fertilization because of a recent wave of publicity in Britain, is the fear that there will emerge a new profession of surrogate mothers — people offering uterine hospitality to *in vitro* embryos. The chances of this happening more often now than hitherto are small, while legal precedent suggests that children born in such circumstances would be the children of their host — and that their later disposal would be governed by the law regulating adoption, including the customary strict interdiction against money changing hands.

Superficially at least, sperm banks and frozen embryos are different. That, however, is more illusion than reality. Given that many couples who at present cannot conceive children naturally are driven to AID, what is more natural than that they should seek to make good their disappointment by looking for some genetic authentication of the sperms they eventually use? Such a system would indeed be far preferable to the present amateurish ways of collecting sperms without pedigrees, but it would of course be unacceptable that sperms should be sold at outrageous profit and that the identity of their donors should be advertised or even disclosed. For such reasons, and also because genetic authentication requires supervision, legislation for the licensing of sperm banks is plainly necessary. It goes without saying, however, that sperm banks cannot offer those who use them more than a negative bill of good health; to say that some known genetic defects are absent is not the same as to promise that the particular combination of paternal genes that meiosis had provided in a sperm will be thoroughly welcome.

Similar arguments limit the likely uses of frozen embryos. The bizarre uses of the technique to propagate an endless succession of children from the same parents, often long dead, will run foul of the unwillingness of women to give hospitality to other people's children (and of the long-established unwillingness of physicians to assist them). But the technique might help to avoid maternally transmitted defects, and should for that reason be welcomed in the rare cases in which it is likely to be used. If ethical committees wish to brood about something tangible, they should worry about cloning — still some way off, but no longer out of sight. The trouble is that such a technique may seem to some to offer means of eugenic improvement justifying all kinds of clandestine stratagems — and which are likely, in the end, to be disappointed by the genetic defects well recognized in amphibian cloning. The best safeguard, for the time being at least, is to require that people engaged on such experiments should discuss the implications of their projects with a suitably independent laboratory committee.

Washington Editor, *Nature*

Ms Deborah Shapley has been appointed Washington Editor of *Nature* in succession to Mr David Dickson, who is leaving the journal at the end of March, after four years in the post. Ms Shapley, a graduate of Harvard University, has worked for the MIT alumni journal *Technology Review* (1968–71) and *Science* (1971–79). She is at present Guest Scholar at Resources for the Future Inc. in Washington, DC after three years at the Carnegie Endowment for International Peace; she is completing a book on Antarctica on a project supported by the Carnegie Endowment.

Nature is looking for a second member of staff to join the Washington office, primarily as a reporter and to commission various contributions to the journal. A formal advertisement will appear next week.

Down with airline cartel

The collapse of Laker Airways should provoke an outcry against government interference.

The benefits of a new technology cannot be assessed in the abstract, but only in the marketplace. This is the conventional wisdom. Whatever ingenuity is lavished on, say, the improvement of mousetraps, an innovation can be counted a success only if its performance and price command a sale among those seeking to rid themselves of mice. For mousetraps and similar products, the primacy of the marketplace is well-established. In other fields of technology, however, all governments choose to suspend the rules



in their own narrow and political interests. They habitually give national manufacturers preference in much of their own purchasing of technological products, from computers to power stations. The result is that the potential benefits of technological advance, and especially potential reductions of costs to ultimate consumers, are needlessly and often scandalously diluted. And scandal is nowhere more rampant than in the airline business, where the potential benefits of a beneficent aeronautical technology are watered down by the insistence of national governments that the places to which aircraft fly should be decided not by the marketplace but by negotiations among diplomats, and that airline fares should be decided by an international price-fixing cartel organized by the United Nations, the International Air Transport Association (IATA).

The only comfort in the unfortunate financial collapse at the weekend of the independent British airline Laker Airways is that the folly of governments' attempts to regulate the world's airlines may now be more widely recognized. Laker Airways, not a member of IATA, has won its reputation in the past five years by offering cheap fares on various transatlantic routes from the United Kingdom. Its attempts to bust the international cartel on routes between Britain and the rest of Europe were, however, frustrated by the unwillingness of governments other than the British to expose their national airlines to competition. While the airline's collapse is probably as much a consequence of commercial overoptimism as of unrealistic expectations that it would be allowed to continue to expand, the suspicion remains that it would not have run into trouble if the international airline business were, in the commercial sense, a business.

The difficulty lies in the widespread assumption by governments that a sovereign government without an airline is like a baseball team without a stadium — incomplete and unconvincing. For how would it seem, in the domestic newspapers, if a president or a prime minister were seen alighting in some foreign capital from an aircraft operated by some carrier other than the national airline? Worse still, how could those lined up to receive such a personage be expected to take the visit seriously if he or she were seen to be a mere fare-paying passenger? (The calculation that since many of these journeys are made with the objective of borrowing money, and that ostentatious extravagance might in the circumstances prudently be avoided, is usually overlooked.) One result is that most governments shoulder without too much complaint the direct subsidies their airlines cost (but the United States government provides hidden subsidies instead). Another is that, to ensure that their national airlines have something to do when not transporting dignitaries about the world, governments assume the right to negotiate strictly bilateral agreements with each other to regulate the numbers of flights by national airlines that there should be. Then, to limit the subsidies they must provide, governments agree that their national airlines should rig the prices that they charge.

To be fair, both the British and the United States governments have recently been trying to break free from this pattern. Within the United States, the consequences of substantial deregulation of domestic flights in the past two years have been dramatic, both in providing cheaper fares and in showing which among the competing airlines are the weak and which the strong. In Britain, the government has allowed small and sometimes new independent airlines to operate new routes and has also encouraged independent carriers such as Laker to seek business abroad. Even if, however, a substantial part of the stock in British Airways is eventually sold to the public, the existing apparatus of horsetrading for routes and price-fixing for fares will remain. For, as things are, the restrictive practices now operated by governments and their national airlines serve one practical purpose — they protect governments from themselves and from each other.

So long as most airlines are supported financially by some public and thus supposedly bottomless purse, price-fixing is defensible. Without it, there would be nothing to prevent airlines in collusion with their governments from openly selling airline tickets below the full cost of servicing them, decreasing its own

financial loss by making fuller use of its aircraft and, in the process, transferring the loss to some other carrier. The snag, of course, is that those who rig the prices are the national airlines, with no conspicuous interest in competition. Bilateral agreements on which airlines should be allowed to fly which routes should in principle be unnecessary if fares have been fixed correctly, taking full account of the costs of servicing capital as well as of flying aircraft.

Not least from nostalgia for the Laker enterprise (which may yet be rescued), the time has come when this bumbledom should be replaced. Regulations, unfortunately, cannot be entirely set aside, for international air transport is socially and commercially too important to be exposed to the risk of commercial cartelization, potentially as damaging as that now operated by governments. But the present regulation of air transport could and should be replaced by a system consisting of very little more than a set of common rules by which competition is permitted.

The first essential ingredient of such a system is that competition on routes and fares should be freely allowed between airlines provided that they do not enjoy the support of governments, direct or indirect. Common standards of safety would be necessary (as at present), but an airline able to pay its pilots and other people less than its competitors should be entitled to charge lower fares. Arrangements would be necessary to ensure that airlines did not subsidize competitive routes or some categories of passengers at the expense of others, but such tasks are familiar and well within the competence of accountants.

But how, if governments are wedded to the concept of a national airline, can such a state of grace be reached? Fortunately, at least some governments seem now to be disenchanted with the need to hand out subventions every year. Why should they not club together to put the airline business on a rational footing, using the existing apparatus of restriction and pricerigging to regulate their dealings with the airlines of more backward states? Even if only Britain and the United States were able to make a deal along these lines, a telling example would be provided for the others. But the natural place for an agreement is within the European Community, which has been talking about the issue for a decade to no effect.

New budget, no change

Science does well, on paper, in the US budget for 1983. But the promise is paper-thin.

President Ronald Reagan's second budget (or is it his fifth?) is, as predicted, kind to science but, unexpectedly, beastly to the conservative aspirations of the President's supporters. Although there will be many disappointments in the proposals published at the weekend, many of the obvious ways in which economies might have been sought have been avoided. The new (and much delayed) accelerator at Brookhaven is not permanently scrapped but put into suspended animation. The basic research programme supported by the budget of the National Aeronautics and Space Administration survives more robustly than seemed likely only a few months ago, marred only by the folly of the decision that there will be even less support for attempts to make sense of the data existing and new which spacecraft will collect. Similarly, the chief sources of support for research at United States universities — the National Science Foundation and the National Institutes of Health — are to be protected from the consequences of inflation. If only (but see page 449) the Department of Defense can spend some of its extra money in the universities, the research community will be in good shape.

The snags are less easily defined. Cutting back on student support will put more severely to the test than in the past few months the unproven hope that students will, when pushed, work their way through every course of study — and may be disastrous. Even more seriously, the cavalier decision that budgets need not be balanced, with its inflationary and international consequences, may so undermine the economic well-being of the United States that the formal protection of research will count for nothing.

DNA panel has second thoughts

Mandatory rules not yet to be abandoned

Washington

Bending to political realities, the Recombinant DNA Advisory Committee (RAC) to the US National Institutes of Health (NIH) has backed away from its support for a proposal that guidelines covering NIH-sponsored research using recombinant DNA techniques should be made voluntary, and that research institutions should no longer be formally required to establish safety committees to ensure that the guidelines are observed.

At its last meeting in September, the members of RAC decided to support the publication for public comment of the proposals for major revisions to the guidelines that would essentially transform them into a voluntary code of conduct.

However, in the light of the comments received — and in particular of warnings that if the guidelines become voluntary states such as California are likely to introduce their own more restrictive regulations — RAC on Monday adopted a compromise formula that would streamline the containment requirements for different types of experiments, but retain their mandatory aspects. Two proposals had been put out for comment. One, a revision of amendments originally proposed by Dr Alan Campbell of Stanford University and Dr David Baltimore of Massachusetts Institute of Technology, would eliminate all the mandatory requirements of the guidelines, including the sanction that non-compliance could lead to a loss of NIH funds.

The alternative proposal, submitted by RAC-member Dr Susan Gottesman of the National Cancer Institute, would retain the guidelines as a required procedure for those receiving NIH grants, including the requirement that certain experiments be reviewed either by RAC or by local Institutional Biosafety Committees (IBCs). The containment classifications, however, would be reorganized, and containment requirements for some classes of experiments would be made less stringent.

The potential consequence of transforming the guidelines into a code of practice for community opinion at local and state levels dominated the discussions.

Most informed scientists who had sent in written comments expressed strong support for making guidelines voluntary, arguing that risks once thought plausible had now been shown to be either remote or non-existent. Dr Paul Berg of Stanford

University, for example, one of the three signatories of the original letter suggesting a moratorium on rDNA research, wrote that he believed the guidelines "are now dispensable".

The institutional response, however, has been different. Many local IBCs, those in universities and in private industry, told NIH that they were not experiencing major difficulties in implementing the guidelines as they now stood. Several warned that the absence of mandatory federal guidelines would provide an opportunity for the introduction of a patchwork of individual laws.

The Industrial Biotechnology Association, for example, which represents a number of small biotechnology companies such as Cetus and Genex, wrote that "one significant reason for adherence to a uniform system of federal guidance and overseeing is our belief that such an approach is more compatible with commercial development and the benefits it brings to society than would be a system of varying local requirements".

If there was an unexpected consensus at Monday's meeting that a strong federal presence in regulating rDNA research

remains desirable, there was less agreement on whether the potential hazards of such research should be treated differently from related areas of biomedical research.

Some members of the committee, arguing the lack of substantial evidence of additional hazard, suggested that this was a reason for adopting uniform practices by making guidelines voluntary. Others, however, used the same data to urge that the experience of RAC should be used as a basis for encouraging a similar approach to research with other organisms.

After hearing the arguments for both proposed revisions, the members of the committee voted by 17 to 3 to adopt those put forwards by Dr Gottesman as the basis for revising the guidelines, rather than those which had been informally endorsed at the September meeting. It also agreed to set up a small working group to revise the specific recommendations which she had put forward to change the current classifications, and to present its conclusions to the director of the National Institute for Allergic and Infectious Diseases, Dr Richard Krause, who will decide what changes to the guidelines will be introduced.

David Dickson

Budget protection for research

Washington

The United States scientific community has done relatively well out of the budget proposals which President Ronald Reagan submitted to Congress on Monday for the fiscal year 1983, which begins on 1 October.

Overall, the President is requesting an increase of about 10 per cent in expenditure on research and development. With an officially-estimated inflation rate of 6.5 per cent, the Administration is claiming that this would represent a real growth in research and development of about 3.5 per cent. The same pattern of growth is being suggested for the basic research budget, scheduled to increase by 8.8 per cent to a total of \$5,821 million.

In both instances, the major contribution to this growth has been increased spending on military research, which rose 25 per cent between 1981 and 1982, and is scheduled for a further growth of 18.9 per cent next year.

Where these increases reflect Mr Reagan's election campaign promises to boost United States military strength, another promise is reflected in moves to reduce substantially federal sponsorship of energy research, on the grounds that this should, where possible, be made the responsibility of the private sector.

The most dramatic reductions are proposed in solar research and other renewable energy sources, such as hydro-power and geothermal, scheduled to come

Recommended budget obligations for fiscal year 1983

	Research and development		Basic research	
	Total	Increase	Total	Increase
	(million \$)	1982-83(%)	(million \$)	1982-83(%)
Department of Defense	24,469	18.9	781	16.0
National Aeronautics and Space Administration	6,513	12.1	682	17.6
Energy Research and Technology Administration*	3,917	-13.3	741	14.5
National Institutes of Health	3,533	3.1	1,897	3.1
National Science Foundation	1,033	7.5	984	7.9
US Department of Agriculture	838	3.8	359	8.1
Environmental Protection Agency	230	- 27.4	10	- 50.0
Other agencies	2,464		367	
Total	42,997	10.7	5,821	8.81

*Previously Department of Energy, intended to become part of the Department of Commerce.

down from \$248 million in 1982 to \$83 million in 1983. Energy conservation research would be reduced even more drastically, from \$144 million to \$18 million; both proposed cuts are expected to be challenged in Congress.

Another agency which has been the target of criticism from the private sector and which would also see its research budget cut under Mr Reagan's 1983 proposals is the Environmental Protection Agency, down from \$317 million to \$230 million.

Other research agencies, however, have done quite well, reflecting a broadly-based acceptance within the Administration that strong federal support for research and development is a legitimate claim on the public purse.

The National Science Foundation, for example, is listed for a budget increase of 7.5 per cent, with considerable real growth in areas such as computer research (up 14 per cent) to \$29.3 million, earth sciences (up 17.8 per cent) and mechanical engineering (up 13.7 per cent). The Administration has relented slightly in its efforts to cut back support for the social and economic sciences, having initially requested \$10 million in the 1982 budget, but now supporting a figure of \$17.8 million in 1983, the same level to which the 1982 budget was raised by Congress. However, it is sticking to its previous determination to eliminate virtually all science education activities sponsored by the National Science Foundation, arguing that education at the school level should remain primarily a responsibility of the states and local school boards.

The National Institutes of Health are scheduled for a modest 3.1 per cent increase in its research budget, slightly less than the anticipated rate of inflation. Unlike last year, the Administration is not seeking to eliminate institutional support for research training awards, a move which provoked a major outcry from biomedical research institutions.

Inevitably, budgetary stringencies have caused disappointments. The National Aeronautics and Space Administration, for example, has for the moment abandoned plans to send an orbiting imaging radar to Venus; but the rumoured extinction of the Galileo mission to Jupiter failed to materialize, and space science is scheduled for a healthy increase of almost 20 per cent, including full funding for the gamma-ray observatory, as well as sustained support for the space shuttle.

Dr George (Jay) Keyworth, the President's Science Advisor, said on Monday that he was "delighted" with the budget increase for science. He said that the physical sciences, once in jeopardy, had been spared during the review process to enable increased utilization of existing particle accelerators. "Next year", he said, "there will be more effort to separate the mediocre from the excellent in research."

David Dickson

US budget described in outline

Energy support

Washington

Not unexpectedly, the requested budget for the Energy Research and Technology Agency will contain strong support for the Clinch River liquid metal fast breeder reactor. The design and construction of the fast breeder are described as priorities and will receive \$257 million for the initiation of site preparation and continued procurement of parts.

However, work on both the Large Development Plant, started by the Carter Administration as an alternative to Clinch River, and the light water breeder reactor will be phased out, leading to an overall decrease of \$108 million from 1982 in spending on breeder reactors.

There will also be reduced support for fusion research, with the focus shifting from the construction of new facilities to what the Office of Management and Budget describes as "resolving key outstanding physics and technology issues". This will delay construction of the tandem mirror test facility at the Lawrence Livermore Laboratory, a decision which has contributed to the resignation of the head of the Department of Energy's fusion programme, Dr Edward Kintner.

Funds for inertial confinement fusion, until recently treated as a major alternative to the magnetic fusion programme, have also been reduced from \$209 million in 1982 to \$119 million in 1983, again through shifting the focus from the completion of new facilities, such as Lawrence Livermore's Nova device, towards experiments on existing machines.

In contrast to the continued — if slightly reduced — support for research into nuclear fusion and fission, only \$315 million is being requested to support research in fossil, solar, biomass and other renewable energy sources, compared with \$814 million in 1982.

Unrest on loans

If US universities have been relieved at escaping the prospect of heavy cuts in research spending, there is less consolation in the Reagan Administration's proposals to limit its assistance for students by reducing the support for the Guaranteed Student Loan (GSL) programme.

First introduced in 1965, the programme provides loan guarantees and interest subsidies to states, private lending institutions and eligible students. Under the present plans, federal support would increase from \$3,397 million in 1983 to \$3,768 by 1987. Tightening up on eligibility requirements and increasing the costs of obtaining a guaranteed loan will result in savings of \$912 million in 1983, rising to \$2,247 million by 1987.

Loans to about 600,000 graduate students would disappear under plans to reduce by about a third the number of so-

called Pell awards. Graduate students would be restricted to raising loans from the less-heavily subsidized GSL auxiliary loan programme, and lending institutions would be required to pay a higher insurance premium to the government.

The Administration claims that the cuts are justified on the grounds that the primary beneficiaries of the guaranteed loans have been "middle and upper income families", which had as a result been able to put their own savings into investment schemes yielding far higher returns. The universities, however, are worried that tightening up the loan conditions could lead to a severe drop in student numbers.

David Dickson

ISABELLE

Construction work on ISABELLE, the 400 × 400 GeV proton-proton accelerator at Brookhaven National Laboratory on Long Island, New York, will be halted under budget plans for the Department of Energy for 1983. However, development work will continue as an accelerator research and development programme on superconducting magnets.

In general, high-energy physics has done well in the budget proposals. Mr Reagan is seeking an increase of 17.5 per cent in funding to \$429 million, claiming this will allow increased use of existing research facilities, the completion of both the Energy Saver and the Tevatron I and II at Fermilab, and further research and development at the linear collider being planned by Dr Burton Richter at the Stanford Linear Accelerator Center.

Construction on ISABELLE, which started in 1978 and had been due for completion in 1987, has been held up largely due to difficulties with the magnets. Although these now appear to have been solved, the delays have meant extra expense. Last November, a special panel set up by the Department of Energy's High-Energy Physics Advisory Panel to look at the future of US high-energy physics concluded that ISABELLE was still important, but did not place its completion as a first priority in stringent budget conditions.

According to the Office of Management and Budget, the accelerator research at Brookhaven will be related to a "future high-energy physics accelerator project", and "any decision to proceed with this project will be based on overall scientific potential and budget considerations".

Space science

The Venus Orbiting Imaging Radar (VOIR) has been dropped from the National Aeronautics and Space Administration (NASA)'s list of future planetary exploration missions. However, the agency is discussing with space scientists the possibility of launching a

cheaper version of the mission, using real rather than synthetic aperture radar, which could halve the estimated \$500 million cost and might still be launched by the end of the decade.

VOIR was initially proposed by the Carter Administration two years ago for a launch from the space shuttle in 1986, with the launch being merely postponed to 1988 by the Reagan Administration last March. It is the major casualty in NASA's space science budget, which otherwise is in a much healthier state than had been widely feared.

Both the Galileo mission to Jupiter and the gamma-ray observatory, which had been threatened by the Office of Management and Budget, have survived. Efforts to cut back substantially on space science seem to have been frustrated by strong pressure from the space science community, and fears that the cuts could have a traumatic effect on NASA's Jet Propulsion Laboratory in Pasadena, California.

NASA will not be providing any money for the development of the Centaur rocket as an upper stage for the space shuttle, previously suggested as an alternative to the delayed Inertial Upper Stage.

One area to be cut will be the analysis of data from other planetary missions, being reduced from \$61.8 million in 1981 to a suggested \$26.5 million in 1983. NASA officials say that although data will continue to be received and analysed from the Voyager spacecraft, now on their way to the outer planets, it may be necessary to stop tracking or taking data from the Pioneer spacecraft.

Delay for *Explorer*

The National Science Foundation has abandoned plans for a major deep-sea drilling programme aimed at investigating the margins of the continental shelf which was to have been conducted from the converted spy ship, *Glomar Explorer*.

Dr John Slaughter, director of the foundation, announced last Saturday that the Reagan Administration would not be requesting any further funds for the so-called ocean margin drilling programme, which was to have been jointly funded with a number of private oil companies.

According to Dr Slaughter there were two reasons for abandoning the project, first formally proposed by the Carter Administration two years ago: a lack of sufficient funding, given general pressures on the federal budget; and the lack of interest by companies in making the type of investment required to convert the *Explorer* and fully equip it.

The proposal has also been a controversial one in the scientific community, since it would have meant curtailing funds for the *Glomar Challenger*. Dr Slaughter said that a decision will be made next summer on whether to refurbish the *Explorer* as a replacement for *Glomar Challenger*.

David Dickson

US defence research

Signs of doubt

Washington

The Reagan Administration is proposing in its latest budget recommendations to Congress (see p447) that there should be an increase in the amount of university-based research sponsored by the Department of Defense in the fiscal year 1983 beginning on 1 October. But the universities themselves are ambivalent about this promise.

Many universities view Defense Department support as a necessary substitute for other federal funds whose sources are rapidly drying up. However, increased military spending on campuses is beginning to act as a catalyst for protest groups opposed to the Administration's policies.

University administrators seem to have fallen in with recent moves by the Department of Defense to increase support for basic research, while fearing some of the restrictions that this money may bring with it. Although many universities still insist that no classified research is carried out on campus, academic leaders have been quick to reassure Congress that little remains of the anti-military sentiment of the 1960s.

Dr Alan Bromley, for example, professor of physics at Yale University and president of the American Association for the Advancement of Science, told the House of Representatives science and technology subcommittee last week that the time to rebuild bridges between the Pentagon and the university research community was "long overdue".

Yet the enthusiasm for increased military support of university research is far from unanimous and there have already been rumblings of protest:

- 170 people from the University of California were arrested last Monday after demonstrating outside the Lawrence Livermore National Laboratory — run by the university on behalf of the Department of Energy — in protest at the laboratory's research on the design of nuclear weapons.

- At the University of Michigan, the students' union has hired a historian to analyse the Defense Department's support of research in the university and in local companies.

- A demonstration was held last summer at the University of Wisconsin's Madison campus over a visit by army scientists to the university's Mathematics Research Center, sponsored by the Department of Defense, to talk about military needs in mathematics. A group has since been formed to study the university's links with the Department of Defense, and a small organization has been set up to stimulate and coordinate efforts by similar groups elsewhere.

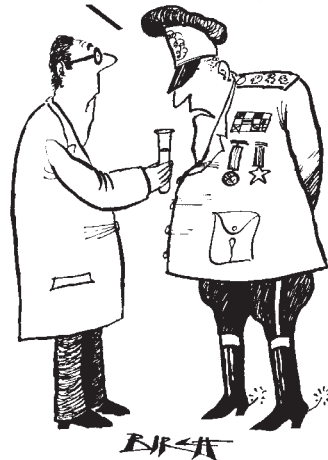
- At the Massachusetts Institute of Technology, about 1,000 people held a

demonstration when Vice-President George Bush spoke to the institute's board of trustees. Several study groups have since been holding regular meetings on military topics, and 30 faculty members have just launched a disarmament project for the spring term.

- There have been protests at Rutgers over a statement by its president, Dr Edward J. Bloustein at a congressional hearing last year that he would be prepared to consider lifting the current ban on classified research on the campus.

- Last November, "teach-ins" were held at 148 colleges and universities across the country on the arms race. The meetings were coordinated by the Boston-based Union of Concerned Scientists, which claimed that they had been the largest anti-war demonstrations since the Vietnam era.

**"VERY GOOD —
WHAT DOES IT KILL?"**



Nobody suggests that these isolated protests represent a significant national movement — at least not yet. Several factors have produced a less fertile environment for protest than the 1960s, ranging from the more conservative outlook of most undergraduates to tight research budgets that can lead to a muting of criticism of the sources of research support.

At the same time, some see as significant the fact that each individual incident has taken place on campuses where there were fierce clashes over military research in the 1960s and early 1970s.

University representatives lobbying the Pentagon for more research funds in Washington tend to play down the potential campus disruption. Even the protesters agree that, although anti-military feeling on campuses has grown rapidly over the past two years, it has yet to coalesce into a major source of opposition.

But the military is still treading warily. When the Defense Communications Agency agreed to sponsor a National Science Center for Communications and Electronics — largely sponsored by the private sector — to stimulate science and engineering education, it chose to concentrate its initial efforts on high schools rather than universities.

Building links with the academic

community "could be a very delicate issue" the agency's director and the principal sponsor of the centre, Lt Gen. William Hilsman, said in a recent interview, stating that the schools had been chosen because they were less likely than universities to view the military interest in education as a threat to academic freedom.

Meanwhile the universities themselves are hoping to receive some guidance from a report recently completed by the Defense Science Board for the Department of Defense. The report is expected to recommend the development of clear guidelines to separate basic research from that which may be of a more practical nature — and hence require special protection and treatment.

David Dickson

British university crisis

Cuts with tears

British universities, still in chaos over how to meet cuts in government income over the next three years, were relieved last week to learn that they are to be compensated for payments made to academics who must lose their jobs. A letter from the University Grants Committee set out the terms under which they will be reimbursed for payments made to redundant staff. Essentially, the compensation scheme is that devised by the Committee of Vice-Chancellors and Principals and blessed by the Secretary of State for Education and Science a week earlier.

The approved scheme allows redundant academic staff below 50 years of age to claim one month's pay for each year of service plus one month's pay for every year of service in excess of five years or after their 30th birthday, whichever is the later. Staff over 50 will be entitled to compensation under the universities' scheme for premature retirement introduced a year ago. The grants committee says that it will not meet claims for compensation that are more generous than those now approved, which begs the question of who will pay if the courts decide that higher awards are just.

The universities' relief will also be tempered by other provisos spelled out in the committee's letter. Full reimbursement will be made only where universities convince the grants committee that redundancies are made in the interests of

economy and that resulting vacancies will not be refilled. Redundancies should also be "consistent with academic planning", a vague phrase thought to give the committee some come-back if universities fail to comply with its advice on general policy.

In the meantime, the end of January deadline by which all universities were to have submitted outlines of how they plan to adapt has passed. Most have sent the numbers of staff they plan to lose by the end of the next academic year, on which basis the grants committee will allocate the £70 million already set aside for helping with restructuring. It seems to be accepted that the sum will be inadequate to settle all claims, although the grants committee has not yet totted up those so far submitted. It is hoped, however, that the government will make more money available by the beginning of the following year.

Only about half the universities have managed to submit returns on how they plan to restructure operations. The grants committee will be looking at the returns later this month, when it will decide whether to reallocate money to those universities that have asked to be reconsidered. The committee is unwilling to say whether the returns suggest that the universities are following its advice, except that one or two of them look "a bit odd".

Some universities seem to have managed to get away with fewer compulsory redundancies than was originally expected. The University of Leicester, for example, plans to make none. The Association of University Teachers claims that success in minimizing compulsory redundancies may reflect the efforts of its members in putting forward alternative strategies for coping with the cuts.

Judy Redfearn

Soviet dissidents

Helpers divided

Scientists campaigning for academic freedom and civil and human rights for their less fortunate colleagues cannot confine themselves to a single cause or campaign. This was the consensus of opinion at a one-day seminar at University College, Oxford, on 7 February, organized by the Scientific and Medical Committee for Soviet Jewry.

The main guest was Dr Mark Azbel, from the University of Tel Aviv, himself a former refusenik and convenor of the Moscow Sunday Scientific Seminars for Refuseniks. Dr Azbel, like most Soviet Jewish activists believes that a clear distinction should be made between the Jewish emigration movement and the dissident movement in the USSR. Any confusion between the Jews, whose only wish is to leave the Soviet Union, and the dissidents who would prefer to stay there and reform the system from within, is, he believes, dangerous to both movements, allowing the Soviet authorities to accuse the Jews of subversion and dissidents of

Small growth fund

The fund established by the Association for Research into Restricted Growth as a memorial to Mary Lindley, an assistant



editor of *Nature* who died in May 1981, has now reached £700. The group intends to use the money to help young people suffering from restricted growth in problems arising during their early education. Further donations should be sent to the Treasurer, Pam Worsfold, 8 Cotswold Avenue, Chelmsford, Essex.

being agents of international Zionism.

The other participants, however, felt obliged to disagree with him. Professor Paul Kessler from the Collège de France, who spoke on "Visiting Refuseniks in the Soviet Union", said that he could not in conscience, while on a visit to Moscow, visit refuseniks and refuse to visit dissident scientists who had also, though for other reasons, been expelled from their jobs. Dr Louis Cohen, executive secretary of the Institute of Physics, speaking on "Transnational science as an influence on Soviet science policy", talked not only of the work of his own institute which in 1978 had sponsored the parallel trial in defence of Dr Yuri Orlov, but also the valuable work of the American Association for the Advancement of Science, primarily on behalf of victims of oppression in South America and also for other human rights causes, including the refuseniks. Dr Gary Low Beer, who is both head of the Scientific and Medical Committee for Soviet Jewry, and a prominent campaigner against the political misuse of psychiatry, put the point bluntly and pragmatically. One cannot expect one's colleagues to campaign for a human rights cause in which one feels a special interest, if one is unwilling to reciprocate for the causes they favour.

In reply, Dr Azbel explained that he did not want to discourage scientists who wished to campaign for dissidents. Everyone, he said, must choose his own cause. But it was better to campaign for only one person at a time, rather than several in parallel.

His support of this line of action seemed inspired not only by practical considerations but by the Yiddish proverb that to save one life is equivalent to saving all humanity. The seminar participants, however, applauded him warmly, but clearly still favoured a unified stand by the scientific community as a whole, on all abuses of human rights and academic freedom, wherever they occur.

Vera Rich

Correction

In the note in News and Views by Dr R.J. Wilson in last week's *Nature* (4 February, page 369), the third paragraph should, except for a misunderstanding in the *Nature* office, have referred to the work of Professor David Weatherall and his colleagues in the Nuffield Department of Clinical Medicine at the University of Oxford; the article describing this work will be published in *Nature* shortly.

Polish universities

On hold again

Student protest continues in Poland, in spite of the stern controls on university life imposed by General Jaruzelski's Military Council for National Salvation. Plans for the opening of undergraduate courses at the University of Warsaw were shelved again last week, following the widespread distribution of an underground leaflet appealing to students elsewhere to support the Poles' right to academic freedom and the restoration of the banned Independent Students' Association (NZS).

NZS, the leaflet pointed out, was legally recognised by the Lodz accords which concluded five weeks of student protest last spring. Almost all the students who held office or played an active part in this legal organization are now, the leaflet said, interned.

In Gdansk, the already severe restrictions on student life have apparently been tightened after a 3,000-strong demonstration on 30 January was broken up by riot police. The local newspaper, which was only allowed to report the event several days later, said that out of the 205 persons detained, 40 were students and 56 were schoolchildren. According to the government's press spokesman, Jerzy Urban, stricter controls have also had to be imposed at Wroclaw Technical University following a student protest march.

Wroclaw is a sensitive area; last autumn, when a young mathematics lecturer and Solidarity activist, Kornel Morawiecki, was charged with including provocative and anti-state material in internal union publications, the rector and academic council spoke out strongly in his defence. Another Wroclaw mathematician, 68-year old Dr Tadeusz Koszecki, is reported to have died of a heart attack while attempting to protect students during a police raid on 23 December.

Expulsions of students and the dismissal of academics unwilling to take the oath imposed by the political "verification" process are reported across the country. Unconfirmed reports state that the expulsion rate at the Academy of Mining and Metallurgy in Krakow is as high as 35 per cent — which bodes ill for the future of Poland's two major industries, for which the academy is intended to supply experts and research consultants. **Vera Rich**

Euratom treaty

Call for change

Brussels

The Euratom treaty, signed by the European Economic Community's six founder members in 1958, is in urgent need of revision, asserted European Commissioner for Industry and Energy, Vicomte Etienne Davignon, at a press conference on 4 February. The Euratom treaty

is an instrument setting up a common market for nuclear energy. It is intended to guarantee common safety standards and safeguards and to ensure that all nuclear energy users obtain a regular supply of nuclear materials. Under the treaty, the European Commission has the exclusive right to conclude supply contracts.

The economic advantages of nuclear power are once again argued in this provision policy paper. The present 16 per cent of EEC's total electricity production created by nuclear power equalled 56 million tonnes of oil equivalent in 1981. With the planned capacity increase of 69 megawatts by 1990, nuclear energy could supply as much electricity as coal and save imports of 150 million tonnes of oil equivalent.

The Commission estimates that electricity from nuclear energy is between 30 and 90 per cent cheaper than that from coal, and compared with petrol nuclear power is 100–150 per cent cheaper. Although the investment costs are high, nuclear fuel costs are negligible compared with coal and oil. And Davignon considers the nuclear option to have other advantages supplying a bonus in jobs and a high level of technology. More detailed economic studies are awaited.

In order to boost investment, the Commission wants Euratom loans doubled. Credit worth 800 million European currency units (approximately \$800 million) has so far been taken up out of the ceiling of 1,000 million fixed in 1977. The new ceiling would then be 2,000 million.

More common efforts are called for from the member states in the storage and reprocessing of irradiated waste. This could involve the establishment of EEC reprocessing companies. In addition, Davignon proposes an end to electricity subsidies, which distort competition among power supplies.

The essential element in the new strategy relates to the supply of nuclear materials. Here judicial changes to the Euratom treaty are envisaged which might mean dismantling the Commission's exclusive trading rights and leaving Brussels with the task of ensuring that there is discrimination towards purchasing countries. By this means, Davignon hopes to stimulate competition among suppliers such as the United States, Canada and Australia with which supply contracts already exist. The Community would then only guarantee that the fission materials would be used exclusively for peaceful purposes.

To reassure public opinion on nuclear safety, the Commission's proposals provide for a more open policy and more information. The system of technical controls and safeguards needs to be updated together with a "concentration of powers". Much the same was recommended in a report by the Belgian Member of the European Parliament, Anne-Marie Lizin (see *Nature* 21 January, p. 179).

Jasper Becker

Laboratory animals

Culture sought

A multi-laboratory research programme organized by FRAME (Fund for the Replacement of Animals in Medical Experiments) to investigate alternatives to animal toxicity tests is being backed by several established drugs and cosmetics manufacturers. Of the £240,000 needed to support the programme, £180,000 has already been guaranteed. The programme involves the collaboration of four laboratories: the Huntingdon Research Centre and centres at the University of Surrey, the University of Nottingham and St George's Hospital Medical School in London.

Although there have been isolated projects on toxicity testing, this is the first in the United Kingdom to link the work of several laboratories. The aim is to scrutinize a common list of chemicals, some toxic, using tissue cultures rather than animals.

The programme is the product of a two-year study by FRAME's Toxicity Committee who will assess the feasibility of non-animal methods of toxicity testing and report in November 1982.

FRAME's board of trustees numbers only four, of whom two are scientists: Dr Michael Balls of the University of Nottingham and Dr Peter Hodges, a senior scientist with Pfizer. Although still a small organization, FRAME seems to have acquired some influence since its inception in 1969. A group of 40 Members of Parliament, led by Joan Lester, has pledged itself to promote alternatives to animals in experiments. Industrial sponsors for FRAME's research programme include Avon Cosmetics, Rimmel International, Bristol-Myers and Pfizer.

FRAME's objectives superficially conflict with the interests of the companies, which at present market their products with a degree of confidence in protection from legal proceedings for negligence because of tests using animals. Their support for the new research programme seems to have been stimulated by the cost of animal screening tests and the growth in public concern for the welfare of laboratory animals.

Thus Avon Cosmetics, which is supporting one of the four research groups says that its interest in alternative methods of toxicity testing is based on ethical considerations. Pfizer says that it has incurred "significant expense" in making senior staff available for internal studies of alternatives to animal laboratory tests, and describes FRAME as its chief British channel for this kind of work.

The Royal Society was urged at a recent meeting to set up an "ethical committee". Its *ad hoc* Committee on Animal Experiments is likely to decide on the terms of reference and chairmanship of such a working party later this month.

Jane Wynn

CORRESPONDENCE

Original life

SIR — David Dickson has quoted (*Nature* 14 January, p.87) from Judge Overton's ruling against the creationists in Arkansas. Among these we find "Although the subject of origins of life is within the province of biology, the scientific community does not consider origins of life a part of evolutionary theory". . . "The theory of evolution assumes the existence of life and is directed to an explanation of *how* life evolved. Evolution does not presuppose the absence of a creator or God. . . ." Now, these statements show that much of the creationist attack on the synthetic theory of evolution is irrelevant. It would be a pity if that short-term goal contributed towards a long-term predisposition in the minds of the scientific community to continue excluding the origin of life from evolutionary theory. That would not be in line with the views of some important evolutionists who contributed to both disciplines. Nevertheless, a bias against considering origins exists in the minds of many evolutionary biologists — I discovered for example that my textbook *Evolutionary Biology* (Holt, Rinehart & Winston, New York, 1972) was not favoured by some teachers because it has a prominent chapter on the origin of life.

The evolutionary process is represented at the molecular level on the one hand and encompasses cosmogony on the other. At some point a theoretical framework covering the entire range of that process will become necessary. Presumably the evolution of molecular systems leads to the origin of life just as the evolution of living systems leads to the origin of ecological systems. Individuals need not become proficient students of all these subjects, but it is quite another argument for an important legal document to declare that part of this subject matter is not considered proper by important exponents of the study of evolution, when that is only the provincial bias of some. Such statements are potentially stultifying and to be regretted by those who otherwise rejoice in Judge Overton's document.

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All in the Book

SIR — That anyone writing from the evolutionists' standpoint is willing to expose such crass ignorance of the creationists' case as does Jon Marks¹ is almost beyond belief. It is much to be hoped that the tone, let alone the substance, from any creationist writing about evolution would not expose such hostility.

May I answer only the three biblical points raised? *Leviticus* 11 v. 19 is not taxonomic but gastronomic; (and very prudent at that) it does not attempt a classification of mammalia. *Luke* 23 v. 43 has the comma incorrectly placed, a point of punctuation well understood over the past four hundred years. *Genesis*, in respect of the fourth day, as taken up by Origen and later by Voltaire, is explained by Wiseman². It is, I regret, open to both sides of this argument to accuse the other of fraud and obscurantism, but nothing useful is served thereby.

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Workers' union

SIR — The letter from ten Russian scientists (*Nature* 24/31 December, p.688) is in part an approach to the WFSW. The federation is indeed concerned with the difficulties that can afflict scientific workers in any country. We have been able, in various circumstances, to resolve or prevent serious problems.

We have been aware for several months that certain requests for exit visas from the Soviet Union have remained unanswered. On 3 November 1981 I wrote to the president of the "Educational and Scientific Workers' Union of the USSR" in Moscow, in conformance with the policy of the federation first to contact our affiliated organization in the country concerned. I asked to be informed of the nature of the difficulties relating to these scientists. I feel certain that Mme Yanoushkovskaya is making the necessary investigation and that she will soon be able to furnish an explanation.

The list of signatories in the letter published by *Nature* is not identical to that in a letter I had earlier received; two names have been dropped and four added. I infer that the situation has evolved since last year.

J.M. LEGAY

World Federation of
Scientific Workers, Lyon, France

Reagan's right

SIR — The editorial "Reagan's mistake on Soviet sanctions" (*Nature* 7 January, p.1) is disturbing. Science is not now (as it may have been in Davy's time) merely a pastime for scientists. It has powerful consequences for humanity in both peaceful and war-like activities. If all humankind were one big happy family, then free interchange of science would be both desirable and inevitable. But humanity is divided into groups whose objective is not to have cheery little wars with "sweetness and light" in view down the road, but to destroy each other. Therefore, free exchange of science cannot be allowed. *Nature's* reasoning resembles that of "pacifists" in the last two Great Wars who helped the enemy and hindered our own war effort because they hated war. We all hate war, and we're all for scientific freedom and exchange. But that is no reason to give science that we've worked or paid for — our science — to people who will use it directly (in weapons) or indirectly, by improving their efficiency, to destroy other people: Poles, Afghanistans, or us.

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An SOS from a Polish scientist

from a correspondent in Poland

SIR — It was not an eruption of anger as in Hungary, nor was it the movement of a rather narrow group of politically mature intelligentsia as in the Czechoslovakian spring. It was a vast popular movement, in which men of the arts, literature and science found their usual role as articulators of popular demands.

This cleansing process began a few years ago. In 1978 the group *Doswiadczenie i Przyszłość* (DiP, Experience and Future) was formed. This small group of scientists and journalists published a number of important reports, their main message being that the country was on the edge of catastrophe and that the very last opportunity of avoiding it by the introduction of essential social reforms had arrived. The authors expected that if their voice was not heard the economy of the country would collapse, leading to social turmoil and possibly bloodshed.

The wave of strikes before August 1980 proved the accuracy of these predictions, but the decline was halted — or, as we see it now, postponed — by the "social contract" signed in the Gdansk shipyard.

Some of those involved in DiP formed an openly acting *Komitet Porozumiewawczy Stowarzyszeń Twórczych i Naukowych* (Coordination Committee of Cultural and Scientific Associations) headed by the philosopher Klemens Szaniawski. This committee played a leading role in the cultural life of the country, especially in preparing and promoting the new law concerning publications, the law aimed at moderating the omnipotent power of censors.

The most spectacular event organized by the committee was the Congress of Culture chaired by the art historian Jan Białostocki. The congress was planned for three days

starting 11 December 1981. Martial law was introduced at midnight December 12 and the last session could not be held.

Among many important speeches delivered during this meeting the most prophetic was the opening address by the president of the Association of Polish Writers, Jan Józef Szczepański. He compared the congress to the orchestra on board the *Titanic*. This prophecy was soon verified — several of the participants of the congress met during the night in the corridors of Warsaw prisons, and many are there still.

At the end of 1980 the process of revival of all scientific establishments began with a few fundamental demands: freedom of scientific inquiry, promotion of scientists according to merit, the right of a university to decide on its curricula and a significant role for scientists in formulating the scientific and educational policies of the country. The autonomy of universities and of the academy, including election of their officers, was seen as a necessary institutional guarantee of the promotion of these demands and the improvement of scientific activities. Autonomy became the keynote of the whole process of change.

Of course none of these demands is very clever. But it is also not very innovative for citizens to demand to be able to influence the fate of their own country, or to require history, records and information to be truthful and not selected according to the needs of authorities. However, these demands coming after many years of the opposite policy sounded heretical. And behind this policy there were people still possessing considerable power and by no means ready to resign from it.

Continued on page 540

NEWS AND VIEWS

Interferon-gamma: success, structure and speculation

from Lois B. Epstein

ANOTHER milestone has been reached in the interferon story with the publication in this week's issue of *Nature* of a report by David Goeddel and his colleagues at Genentech and NIH of the first successful identification of an interferon-gamma (IFN- γ) gene and the expression of a cloned cDNA sequence for human IFN- γ in bacterial and monkey cells¹. Since the discovery in 1965 of an 'interferon-like substance' in the supernatants of human leukocyte cultures stimulated with phytohaemagglutinin², much progress has been made in defining its cellular origin, range of inducers, physical and chemical properties, actions and methods of purification³. This substance, originally called type II or immune interferon, is now called IFN- γ to distinguish it from IFN- α and IFN- β which were discovered earlier, and are now known to have different cellular origins, physical properties and antigenic determinants.

Lymphocytes produce IFN- γ in response to numerous mitogens, bacterial and viral antigens, allogeneic cells and antisera directed against lymphocyte surface determinants. Although the purification of IFN- γ was initially hampered by the relatively low levels induced and considerable loss of activity during the purification procedure, numerous laboratories throughout the world persisted in their efforts. To date, preparations having a specific activity of greater than 10^7 units per mg have been attained⁴⁻⁶, but microsequencing information is not yet available. Although at first IFN- γ was thought to be larger than the other interferons with a molecular weight of 35,000–70,000, the most recent report indicates that it can exist in 20,000 and 25,000 molecular weight forms⁷.

Initial efforts to define the range of actions of IFN- γ were hampered by the lack of abundant or partially purified material. Many investigators were forced to use crude preparations which contained numerous other lymphokines and factors that affected cell function independently of the action of the interferon. However, with

the development of better methods of purification and the preparation of antisera to IFN- γ ^{8,9} specific activities could be assigned to IFN- γ in a more rigorous manner. It became clear that although IFN- γ differs from IFN- α and IFN- β in its response to low pH, antigenic determinants, host range and cellular origin, it still induces the same enzymes¹⁰ and the same peptides^{11,12}, and shares their ultimate antiviral¹¹, immunoregulatory⁹ and antitumour¹³ actions. To date no unique function which would distinguish IFN- γ as a separate functional entity from the other interferons has been found.

Recently, four laboratories have isolated and translated mRNA for IFN- γ in oocytes¹⁴⁻¹⁷, thus paving the way for future production of IFN- γ by recombinant DNA techniques. The latest achievement by Goeddel and his colleagues gives testimony to the tremendous accomplishments now possible with recombinant DNA technology when multiple resources are integrated into an attack on a specific problem. If one considers the many methodologies and types of expertise required to accomplish this work it is clear that it could only have been achieved in a large industrial research centre.

The cloning of the IFN- γ gene now makes it possible to produce IFN- γ in unlimited quantities and should lead to profound insights into the evolutionary history of IFN- γ , its tertiary structure, the relationship of its primary structure to its function and physical properties, and its genetic control. In the report by Goeddel *et al.* it is suggested that the gene which codes for IFN- γ contains an intron. This is in contrast to the genes which code for IFN- α and IFN- β which contain no introns. Conclusive proof of this must await future studies when the genomic sequence of the IFN- γ gene is determined and compared

with the present cDNA sequence. The results also demonstrate that IFN- γ has a molecular weight of 17,100, consistent with another recent report based on physical measurements⁷, and suggest that higher molecular weights observed by other investigators might have been due to multimeric forms of IFN- γ or to the fact that IFN- γ is glycosylated *in vivo*.

If the primary amino acid sequences of the various interferons are compared, it is apparent that 80 per cent homology exists within members of the IFN- α family and 30 per cent between IFN- α and IFN- β . Goeddel and his colleagues report that their cDNA sequence codes for a polypeptide of 166 amino acids, of which 20 could constitute a signal peptide. The authors have looked for sequence homologies between their IFN- γ and IFN- α and IFN- β and indicate that the only homology noted covering more than three consecutive amino acids occurs at amino acids 15–18 in IFN- γ , and these are found in positions 121–124 in two recombinant forms of IFN- α . I find it intriguing but strange that although IFN- α , - β and - γ have a common range of biological actions such little apparent homology exists. However, in my own comparative examination of the sequence of Goeddel's IFN- γ with that of several recombinant forms of IFN- α I find a definitive pattern of single amino acid homology throughout the entire sequence (see the figure). If one postulates a difference of one amino acid in the length of the IFN- γ sequence between residues 49 and 65 and of the recombinant IFN- α sequence between residues 49 and 66, then 14 positions have identical amino acids and 4 more differ by only the most conservative replacement (Asp for Asn; Leu for Ile; Ile for Leu). Thus there are 12 per cent identical amino acids or most conservative replacements in precise sequence order.

	1	41	42	49	65	68	72	78	84	93	94	96	103	116	117	119	131	133
IFN- γ	Cys	Glu	Glu	Gln	Asp	Ser	Ser	Glu	Phe	Asp	Asp	Glu	Val	Leu	Ile	Val	Lys	Lys
IFN- α	Cys	Glu	Glu	Gln	Asn	Ser	Ser	Glu	Phe	Asn	Asp	Glu	Val	Ile	Leu	Val	Lys	Lys
	1	41	42	49	66	69	73	79	85	94	95	97	104	117	118	120	132	134

Fig. 1 Sequence homology of recombinant IFN- γ and IFN- α . The information is derived from published amino acid sequences for IFN- γ ¹ and IFN- α ²².

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Even more intriguing is the fact that if one examines the model for the tertiary structure of IFN- α and IFN- β proposed by Sternberg and Cohen¹⁸ virtually all the conserved areas shown in the accompanying figure are clustered in the exposed β sheets linking the four roughly parallel postulated α -helical segments of the molecule or on the exposed surface of one α -helical region.

Although chromosome 21 has been shown to control the response to all the interferons¹⁹, recent evidence suggests that the receptors for IFN- γ and the other interferons may be different^{20,21}. Without knowing how any interferon interacts with its receptor or what part of the molecule induces its subsequent effects, it is not clear where these conserved amino acids fit into the picture of interferon-receptor binding and interferon function. I would expect, however, given the probable existence of different receptors, that the various interferons would differ in regions that bind, and that the conserved regions would be important determinants of effector function, in analogy to regulatory and catalytic sites on allosteric enzymes.

There is tremendous interest, enthusiasm and expectation for the future of IFN- γ as an antiviral, antitumour, or immune response modifier. The issue that has attracted the greatest attention is its potency relative to that of the other interferons. In some cases IFN- γ has been shown to be more potent than the other interferons, but there are an equal number of cases which do not substantiate such a difference. Resolution of the question of relative potency of IFN- γ depends on its purification to homogeneity so that it can be compared mole for mole with the other interferons with regard to all their known biological effects. At present all units used to describe IFN- γ are peculiar to a given laboratory, and as such are arbitrary. It is

hoped that an international standard for IFN- γ will be available in the near future, which will permit all the IFN- γ preparations to be compared on a common basis.

Not only is IFN- γ of considerable intellectual and scientific interest but also of tremendous commercial interest as well. This is demonstrated by the large number of commercial, private, or university-based laboratories now devoted to its production in large quantities by conventional or recombinant DNA techniques. The object of many such ventures is to prepare enough IFN- γ for future clinical trials on patients with malignant or infectious diseases or with immunological defects. However at present the amount of information available on which these trials would be based is very limited.

Thus there is much still to be learned about

IFN- γ . The primary, secondary and tertiary structures of IFN- γ induced by various inducers must be determined to see whether they are the same or different. The chromosome which controls the production of IFN- γ , and the nucleotide sequence of the gene on chromosome 21 which controls the response to IFN- γ , must be determined. The biochemistry of the action of IFN- γ and its method of induction at the cellular and genetic levels must be defined. Abnormalities in IFN- γ production or action in various diseases must be sought, and the range of viruses susceptible to its antiviral activity determined. Finally, concerted efforts must be made to determine the manner and degree to which the growth of various human tumours is affected by IFN- γ so that its maximum therapeutic potential may be realized. □

Stem cell regulation in erythropoiesis

from P.R. Harrison

EVER since it was demonstrated using genetically marked cells that all types of blood cells derive from a common progenitor, the haematopoietic stem cell, interest has been focused on trying to define what controls the differentiation of the stem cell into one type of blood cell rather than another and how the relative numbers of blood cells are regulated.

Although it has long been known that the tissue environment has an important influence on the differentiation of a stem cell, the way in which it exerts this influence is highly controversial. The various mature functional blood cells are all derived from relatively small numbers of stem cells by way of a series of intermediate precursor cells which become progressively more restricted (or committed) in the types of blood cells they can produce; large numbers of mature blood cells are then produced by proliferation and maturation of the appropriate committed precursor cells (see the figure). One view of stem cell differentiation sees the microenvironment as having an instructive or determinative role in causing a stem cell to become committed to one blood cell lineage rather than another^{1,2} through mechanisms such as cell-cell interactions or short-range 'inducers'. But critics of this hypothesis have shown that the way in which a stem cell proliferates or becomes committed can be explained in terms of a purely random genetic process^{3,4}, perhaps involving some form of DNA rearrangement or movement of transposons. Other strong evidence against an instructive role of the tissue microenvironment at the commitment stage is the finding that in spleen colonies formed after inoculation of bone marrow cells into irradiated mice the relative

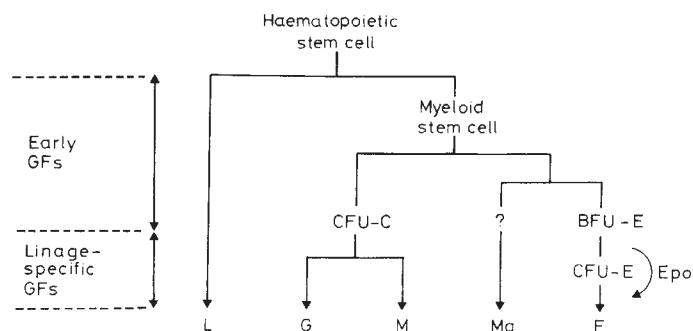
numbers of the various types of the earliest granulocyte and erythroid committed precursors (CFU-C, BFU-E) are strongly correlated, irrespective of colony size (or morphology)⁵. Thus it is argued that the various haematopoietic tissue environments influence the types of blood cells produced purely by facilitating the proliferation of particular kinds of committed precursor cells.

The 'instructive microenvironment' hypothesis now seems to have taken another knock with new experiments reported by Magli, Iscove and Odartchenko in this issue of *Nature* (p.527). They used the classic spleen colony assay for stem cells devised by Till and McCulloch⁶, where cells of interest are injected into lethally irradiated mice and the presence of haematopoietic stem cells is quantified by counting the number of macroscopic colonies formed on the surface of the spleen. After 7 days the spleen colonies consist mainly of erythroblasts, whereas spleen colonies present after 11 days comprise a variety of haematopoietic cell types, including granulocytes and megakaryocytes as well as erythroblasts. It has previously been assumed that, since each colony is derived from a single cell, the changes in the local environment as the colony grows and comes into contact with different parts of the spleen are responsible for changes in the differentiation pattern. What Magli and her co-workers have now shown is simply that the mixed colonies present after 11 days are not in the same positions as the early erythroid colonies, and that only the

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Simplified ontogeny of blood cells. L, lymphocytes; G, granulocytes; M, macrophages; Mg, megakaryocytes/platelets; E, erythroblasts/red blood cells; GF, growth factor; Epo, erythropoietin. CFU-C, precursor cell giving rise only to granulocytes and macrophages; BFU-E and CFU-E, early and late erythroid precursors respectively; the CFU-E is responsive to erythropoietin. Some scientists believe that the B lymphocytes and the myeloid stem cell are derived from a common precursor whereas the T lymphocytes derive from the haematopoietic stem cell independently. Others believe T and B lymphocytes derive from a common lymphoid precursor independently of the myeloid stem cell (see ref. 7). There is also evidence for a common precursor of erythroid and megakaryocyte lineages.



late colonies contain precursors capable of proliferating in culture to form colonies having mixed differentiation patterns (that is, early precursor cells of some kind, although not necessarily haematopoietic stem cells *per se*). This result rather undermines the argument for an influence of the splenic microenvironment on differentiation pattern during colony growth. Furthermore, since stem cell assays have traditionally been based on early colony counts, it also means that certain conclusions about stem cells will need to be reassessed.

But the question of how the haematopoietic tissue environment facilitates the proliferation and/or differentiation of particular types of committed precursor cells remains open. Much of the progress in this direction has resulted from the development of *in vitro* systems capable of supporting the growth of stem cells on bone marrow feeder layers by Dexter and his colleagues in Manchester and of assays for committed precursor cells of various kinds by Metcalf, Johnson, Iscove, Axelrad and others. It seems that, in general, both the earliest precursor cells and the various intermediate committed cell precursors require growth factors to sustain their proliferation and differentiation (see the figure; and ref. 7 for a recent review). As such factors are often produced by other haematopoietic cells or stromal cells in the tissue environment, then clearly the regulation of haematopoiesis involves complex interacting cell networks.

Does the production of these growth factors explain the effects of the tissue macroenvironment on the differentiation of haematopoietic stem cells? The answer is probably not, according to Dexter⁸. It appears, for example, that purified growth factors cannot mimic the local effects of the microenvironment if the stem cells are separated from the stromal cells by a thin

layer of agar or if they are cultured in diffusion chambers where direct cell-cell contact is prevented. So either cellular contacts are involved as well as the

circulating growth factors, or the essential factors produced by the stromal cells are extremely labile and therefore act over only very short distances. □

The nature of immune response gene defects

from Ronald H. Schwartz

A NUMBER of recent experiments on immune responses *in vitro* have suggested what will seem to some a revolutionary solution to the conundrum of the antigen-specific genetic defects in immunity that are controlled by the immune response (Ir) genes. These genes were discovered in the early 1960s, in the course of experiments on inbred guinea pig and mouse strains immunized with simple synthetic polypeptides¹. The experiments showed that some strains (known as nonresponders) are unable to respond to specific antigens, and that this blind spot is due to a single dominant genetic locus in the major histocompatibility complex (MHC). It is now clear²⁻⁵ that the Ir genes code for Ia molecules — polymorphic two-chain polypeptides expressed, like the other major histocompatibility antigens, on the cell surface. The first real clue to how the Ia molecules might influence the immune response lies in the phenomenon of MHC restriction⁶: T cells can recognize foreign antigens only in association with products of the MHC — the particular product recognized being dependent on the subset of T cells involved in the response. The T cell subset that recognizes Ia molecules is the amplifier T cell subset⁷, which among other things cooperates with B cells in the production of antibodies. These T cells are believed to be activated by foreign antigens presented in the context of an Ia molecule on the surface of a macrophage.

Thus it is clear how in principle an Ia molecule could influence an immune response; but it is not obvious how a particular Ia molecule leads to non-responsiveness only to certain antigens. Two solutions to this problem have been proposed. One, the so-called determinant selection model, is that different Ia molecules possess different abilities to bind antigens, and that non-responsiveness arises from the failure of the macrophage

bearing the nonresponder Ia molecule to bind the antigen for presentation to the T cell^{8,9}. The alternative is that the T cells specific for a particular combination of antigen and Ia molecule are missing in nonresponder strains¹⁰⁻¹². This could be because of the absence of a gene encoding a T cell receptor with the relevant specificity; or it could be secondary to somatic selection of the T cell repertoire. Two kinds of selection have been envisaged. One is negative selection, in which T cell clones responding to self determinants are deleted during the generation of tolerance. The immune deficiency would then arise because a particular combination of Ia molecule and foreign antigen mimicked a self determinant¹⁰. Alternatively, T cells may be positively selected to produce a repertoire in which antigen recognition is restricted to self MHC. In this case, the immune defect would be due to a failure to select for those clones recognizing the non-responder Ia molecule in association with a specific foreign antigen^{11,12}. It is to this theoretical controversy that the experiments reported by Ishii and his colleagues in this week's *Nature* (p.531) are addressed.

Their experiments were designed to eliminate an ambiguity inherent in the results of earlier experiments on the cellular basis of non-response. The usual procedure has been to immunize a hybrid (responder x nonresponder) F₁ animal, and then test its T cells for proliferation in response to antigen plus either responder or nonresponder macrophages *in vitro*. In such experiments, the F₁ T cells always respond to the antigen in combination with the responder macrophages, but not in combination with the nonresponder

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macrophages¹³. This has been taken to mean that it is the macrophage and not the T cell that is defective. But if the defect were in fact due to the absence of certain T cell clones as a result of somatic selection, then one would expect the F_1 to be unable to respond to antigens presented on nonresponder macrophages. Thus the only way to test unambiguously whether the defect is in the macrophage or the T cell repertoire would be by using fully allogeneic nonresponder macrophages to present the antigen.

In order to do this, Ishii *et al.* had to overcome a technical difficulty: normally, T cells would be expected to mount an immune response to the allogeneic macrophages alone, and this would obscure any proliferative response to the combination of allogeneic Ia and antigen. They therefore had first to remove any T cells reactive to the macrophage alloantigens. This was accomplished by stimulating the T cell population with the macrophages in the absence of antigen and killing the proliferating alloreactive T cells by exposure to bromodeoxyuridine and light. The remaining T cell population was then restimulated with the same nonresponder macrophages, but now in the presence of antigen. The interesting result which they obtained was that these T cells could respond to the antigen in association with the nonresponder macrophages. Even if the T cells were obtained from one nonresponder strain and thus could not see the antigen in association with their own macrophages, they could respond to the same antigen in association with the macrophages from another nonresponder strain. These results suggest that a nonresponder T cell population is only blind to the antigen in the context of its own syngeneic macrophages. This in turn argues strongly against several of the theoretical models discussed above. First, if T cells exist which can recognize the antigen and nonresponder Ia molecules, then genes must exist in the species which encode for these receptors either as direct products or as somatic variants. Second, if T cells can respond to

the antigen and nonresponder Ia molecules, then the Ia molecules themselves cannot be defective in binding and presenting the antigen. Therefore, these experiments can be interpreted as arguing against determinant selection models and by default supporting the somatic T cell selection models for generating Ir gene defects.

This is not the first report of nonresponder macrophages presenting antigen to responder T cells. In 1973 Kapp *et al.*¹⁴ demonstrated that nonresponder macrophages could stimulate a helper T cell-supported antibody response from responder spleen cell populations. However, these experiments were criticized because the investigators did not deplete the responding population in alloreactive cells, which might have produced false positive results through complex interactions between lymphocyte subsets and the release of soluble recruitment molecules (so-called positive allogeneic effects)¹⁵. Even in the experiments reported by Ishii *et al.* one has to ask whether the authors have sufficiently depleted the T cell population in alloreactive cells to eliminate all such effects. They demonstrate that the nonresponder macrophages alone do not induce a proliferative response, suggesting by the criterion of cell division that alloreactivity has been removed. However, given the fact that all the strain combinations reported on in the paper produced a response, one has to worry whether residual alloreactivity, such as the release of recruitment factors, still remains. The authors state that they have some negative results using strain combinations with only partial histocompatibility differences, but these combinations might also generate

reduced allogeneic effects.

Nonetheless, the implications of the findings reported in this paper have been supported by recent results from other laboratories. Pierce *et al.*¹⁶ reported that responder T cell populations contain cells capable of helping B cells from nonresponders to make antibody, whereas (responder $\times$ nonresponder) F_1 populations lacked such T cells. Even more striking have been the data obtained with cloned T cell populations. Clark and Shevach¹⁷ have isolated several clones from a responder strain of guinea pigs that respond to antigen presented on nonresponder macrophages from a different strain, but not to nonresponder macrophages alone, or to the same antigen presented on responder macrophages. This last result is analogous to that of the Ishii experiments. The beauty of the cloning experiments is that they totally eliminate the possibility of a false positive allogeneic effect since alloreactive T cells have been entirely removed. On the other hand, the problem with the cloning results published so far is that they only describe a few examples. These could represent rare phenotypes and thus might not be sufficient to explain Ir gene effects, which require the absence or lack of stimulation of whole families of T cells with similar specificities⁷. Overall, however, the data from both types of experiments would seem to complement one another, the clones addressing the doubts about alloreactivity and the Ishii experiments addressing the doubts about population size. Thus it would appear that these collective experiments represent a strong challenge to determinant selection models. It remains to be seen how and if this challenge will be met. □



100 years ago

THE MOVEMENTS OF JUPITER'S ATMOSPHERE

Mr. Darwin describes the bands on Jupiter as "due to the trades and anti-trades" set in motion by the action of solar radiation on the solid body of the planet as are the trade-winds of the earth. Many other eminent astronomers still appear to accept this time-honoured explanation of the phenomena.

Have they reflected on the revelations supplied by the low specific gravity of Jupiter? There is no form of matter with which we are acquainted that could exist at a mean density of about one-fourth of that of the earth, while subject to the enormous pressure due to the mass of Jupiter, unless it were sufficiently hot to render the formation of a solid crust on its surface quite impossible. In order to attribute terrestrial solidity to either Jupiter, Saturn, or Neptune we must invent a new kind

of matter as infusible as platinum, and far lighter than hydrogen, or endow it with absolute incompressibility.

These planets, if composed of any of the chemical elements or compounds known to us, can only retain their low density under the enormous pressure of their masses by the agency of proportionately counteracting heat-repulsion. At and about their centres this may be so far overcome by the superincumbent pressure as to produce solid nuclei.

Assuming the existence of such a central nucleus of Jupiter surrounded by a great fluid envelope, how will it be affected by the gravitating reaction of the satellite, supposing the compression to give it a specific gravity exceeding the mean specific gravity of its envelope?

It will obviously perform an eccentric rotation, or reeling, within the envelope. This motion must be very irregular and complex, owing to the different periods and the varying relative positions of the satellites. The effect of such internal reeling upon the surrounding gaseous mass explains far more efficiently than any possibility of solar radiation, the disturbances indicated by the ever-changing belts and spots of this planet.

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Making muonium in vacuum

from C.J. Batty and G. Marshall

MUONIUM is the name given to the simple atomic state consisting of a positively charged muon and an electron. For atomic and chemical purposes it is aptly described as a light isotope of hydrogen with only about one-ninth the hydrogen mass. However the properties of its 'nucleus' make muonium both interesting and useful in physics and chemistry. Two recent papers describe a novel way of forming muonium which will extend its use to measurements in vacuum.

The muon, identified in 1936 in cosmic ray studies, was one of the earliest of the elementary particles to be discovered. But despite an enormous amount of study and a considerable knowledge of its properties, its existence remains an enigma. Like the electron and the positron, negative and positive muons are weakly interacting point-like leptons and are not influenced by the strong interaction felt by hadrons. The muon differs from the electron essentially only in its mass, which is 207 times that of the electron.

It is relatively simple to produce intense, high-quality muon beams from the decay of pions obtained at some particle accelerators. So it has been possible to make a thorough study of the properties of the muon, and then with this knowledge, to use the muon as a tool for measurements in other fields. Thus the spin and magnetic moment of the muon, together with the fact that its weak decay (with a mean lifetime of $2.2 \mu\text{s}$) into an electron plus two neutrinos is asymmetric or parity violating, have been used to give information on the magnetic fields inside a variety of materials. This technique of muon spin rotation, or μSR in analogy with NMR and ESR, is a subject of intense interest at several laboratories throughout the world. Another application involves the formation of muonic atoms, in which a negative muon is captured in an atomic orbital, effectively replacing one of the electrons. Measurements of the radiation emitted in the subsequent atomic cascade can determine the distribution of charge in the nucleus.

The formation of muonium is fundamental to experiments with muons in fields as diverse as quantum electrodynamics, particle physics, solid-state and surface physics, and chemistry. For most applications muonium can be formed by arresting a beam of energetic positive muons in a suitable solid, liquid, or gas target, in the same way that hydrogen atoms can be formed by stopping proton beams. Near the end of its range, the positive particle loses energy by ionization, and in the low-velocity regime (corresponding to less than about 20 keV for muons) there is a high probability that it will form a neutral muonium atom. In

general the muon will capture and lose an electron many times before it reaches thermal energies, and if the likelihood for the capture process is large compared with that for electron loss as thermalization becomes complete, muonium will be formed. In most chemical and solid-state applications the interaction of muonium with the target material is studied, but this interaction can be a hindrance when studying certain other phenomena. It is therefore of interest to develop an alternative method of muonium production so that measurements can be made on the atom in vacuum.

A group working at the LAMPF accelerator facility in New Mexico has recently reported the observation of fast (~ 10 keV) muonium in vacuum (P.R. Bolton *et al. Phys. Rev. Lett.* **47**, 1441; 1981). The authors allowed an intense low-energy (4 MeV) muon beam to slow down in a polyethylene degrader before it passed through a thin foil target in vacuum. It is predicted, on the basis of proton measurements, that a significant fraction of the muons emerging with energies less than 20 keV will be in the form of muonium.

In order to separate the small neutral muonium component from the charged remainder of the beam, a 5 kG magnetic sweeping field is used immediately after the target foil. The neutral particles travel 160 cm from the target through the magnetic field to a beam stop. The positrons expected from the decay of the muon are detected in an array of scintillation counters and a NaI detector. If the e^+ are from the decay of μ^+ then they should have a characteristic energy spectrum with an endpoint energy of 53 MeV.

This characteristic energy spectrum, superimposed on a flat background of events due to cosmic rays and dominated at energies below 30 MeV by counts due to the electron contamination in the incident beam, has been observed in the experiment reported. The introduction of a few Torr of helium gas into the normally evacuated ($< 5 \times 10^{-5}$ Torr) apparatus causes the muonium signal to disappear due to the ionizing collisions $\mu^+ e^- + \text{He} \rightarrow \mu^+ + e^- + \text{He}$; the μ^+ are then swept away by the magnet, and the background spectrum can be measured. The results show that, for the particular muon beam used, the production rate for muonium is about 3×10^{-4} per incident μ^+ regardless of which of several target foils is used. The conclusions are consistent with proton beam data and with models of the neutralization process.

Estimates of the energies of the muonium atoms indicate that 50 per cent have energies of less than 5 keV and 95 per cent of less than 20 keV.

There are two measurements in particular which would benefit from copious quantities of muonium in vacuum. One is a sensitive search for the conversion of muonium to antimuonium ($\mu^- e^+$) which can be accommodated in some versions of the electroweak theory. The interaction of muonium with matter drastically reduces the expected conversion rate, so that current limits on the process are not particularly restrictive. The other is the Lamb shift in muonium, the energy difference between the $2s_{1/2}$ and $2p_{1/2}$ states of the $n=2$ atomic level. Since both the muon and the electron are leptons and thus have no structure, quantum electrodynamics can predict the Lamb shift very accurately, in contrast to the case for atomic hydrogen. The fast muonium emerging from the foil in the LAMPF experiment should contain a $2s$ component of about one-eighth the total muonium intensity.

This component was also shown to exist in another recent experiment performed by a group from the TRIUMF meson facility in Vancouver, Canada (C.J. Oram *et al. J. Phys. B* **14**, L789; 1981) using a similar muon beam and thin foil target. Muonium produced in the skin of the foil emerged to pass through two successive sets of parallel plates, used to produce high electric fields at right angles to the direction of motion of the atoms. The electric fields will introduce through the Stark effect a $2p$ component to any $2s$ state which may be present. Decay can then proceed from the $2p$ to the $1s$ state by emission of UV light with a lifetime of about 3 ns. The light can be detected by a wavelength shifter and photomultiplier system mounted close to the second set of plates. A clock is started when a muon is incident on the thin foil and is stopped when an event is recorded by the UV detection system.

If voltage is applied only to the second set of plates the time spectrum should show three muon components: an exponential decay due to muon-decay positrons incident on the photomultiplier system; a flat background due to uncorrelated events; and a foreground peak, about 150 ns wide, due to muonium in the $2s$ state decaying in the region of high electric field. The foreground peak should largely disappear when an electric field is applied to the first set of plates, as this would cause the $2s$ state muonium atoms emerging from the foil to decay rapidly to the $1s$ ground state before they reach the second set of plates and photon detector system.

Further experiments are planned to improve the production of muonium in vacuum. In this way, the goals of an accurate determination of the Lamb shift and a sensitive search for the conversion of muonium to antimuonium should be achieved. □

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Is amorphous silicon played out?

from J.I.B. Wilson

IN spite of enthusiasm for amorphous silicon as a means of making cheap photovoltaic diodes, the improvement of the performance of these diodes has recently been slow and difficult. So is amorphous silicon destined to be a false trail in the search for a cheap solar cell? Although some Japanese companies (principally Sanyo Electric Co.) have set up production lines for these solar cells, chiefly for use in consumer products such as calculators, large-scale power conversion requires a better efficiency than the present about 7 per cent. The main barrier to further improvement is the poor photocurrent, caused by the high concentration of imperfections in the material. These trap the free charges produced by light and inhibit their collection by the metal contacts.

Scepticism about the potential of amorphous silicon cells is easily justified. It has always been suspected that an amorphous semiconductor will have unavoidably poor electrical characteristics, even when it is passivated with hydrogen produced by the plasma decomposition of silane gas (SiH_4). So is it possible to improve the electrical conductivity of this hydrogenated amorphous silicon (a-Si:H) only by sacrificing the high optical absorption of the amorphous structure? Although the hydrogen will reduce strain in the structure and reduce the number of unsatisfied silicon bonds, it will not necessarily control the concentration of defects, so that hydrogen content is not in itself a measure of the structural disorder.

Indeed, recent papers^{1,2} suggest that in a-Si:H the structural disorder rather than the hydrogen content controls the optical absorption, especially below the 'bandgap', even though the removal of hydrogen by heating leads to both a deterioration in semiconducting behaviour and an increase in optical absorption. This suggests that if the optical absorption is to remain high, then the electrical behaviour of devices made with a-Si:H may always be limited by intrinsic structural disorder even if the structure is modified with hydrogen or fluorine. This conflict is already accepted in efforts to develop solar cells, and more complicated structures than a simple metallurgical junction have been suggested as ways of increasing the photocurrent without abandoning the simplicity of the plasma technique of depositing semiconductors.

In spite of theoretical estimates of the position in the energy level scheme of particular defects, such as unsatisfied silicon 'dangling' bonds and those produced by silicon-hydrogen groups, the

inverse process of identifying bandgap energy levels with the physical entities to which they correspond has been unsuccessful, largely because of disagreement about the actual distribution of these energy levels within the bandgap. (Only in a pure crystalline semiconductor is this bandgap completely disallowed.)

Since the bandgap levels are those responsible for photoluminescence and photoconductivity, as well as for the control of electrical conduction, it is surprising that there should still be so many uncertainties. One difficulty in the interpretation of experimentally determined distributions is that the surface of the semiconductor (or its interface with an electrical contact) may contribute a substantial number of additional energy levels, perhaps with an energy distribution different from that in the semiconductor bulk. Another is that some experimental techniques are insensitive to any structure in the distribution. Thus field effect measurements (using a metal oxide-semiconductor transistor) give a mid-bandgap density of energy levels of $\sim 10^{17} \text{ cm}^{-3} \text{ eV}^{-1}$, whereas a transient capacitance technique (deep-level transient spectroscopy), which is said to distinguish between bulk and surface effects, has given a surprisingly low value of $\sim 10^{15} \text{ cm}^{-3} \text{ eV}^{-1}$. There is, however, disagreement about the interpretation of this data³.

Moreover, there is one intriguing electrical conductance phenomenon for which the simple one-electron model of the energy levels due to defects appears to be inadequate — the 'Staebler-Wronski effect'. This consists of photostructural changes when a-Si:H is illuminated with a high flux of visible wavelength photons⁴: the electrical conductivity gradually decreases by a small factor, but the subsequent dark conductivity decreases by up to 10,000 times. These changes can be reversed by annealing the sample at $\sim 150^\circ\text{C}$ for some minutes, but there is no distinct temperature threshold. Although similar effects can be produced by adsorption on the surface, this is definitely a bulk effect and is ascribed to a movement of the Fermi level towards the middle of the bandgap. The phenomenon does not occur in heavily doped p- or n-type samples.

An explanation invoking self-trapped excitons has been proposed by Elliot⁵, while a more elaborate model due to Adler⁶ successfully explains variations of the effect in lightly doped samples as well as other experimental observations. Adler proposes that two adjacent neutral dangling bonds (T_3^0) in the predominantly tetrahedral lattice (for not all dangling bonds are removed by hydrogen) are dehybridized from their usual sp^3 character to the (trigonal) sp^2 - p_z form. The transfer

of an electron creates a pair of charged centres, an acceptor (T_3^+) and a donor (T_3^-), but their energy levels within the bandgap are thought to be inverted compared with those of the usual substitutional impurity donors and acceptors.

On Adler's model, interconversion is prevented by a potential energy barrier, for the charge centres have different bond angles within the lattice. On this view, the decrease in photoconductivity and the shift in Fermi level observed in the Staebler-Wronski effect are produced by the asymmetric trapping of free charges at these energy levels, with a consequent conversion of neutral donors to acceptors. The increased concentration of acceptors causes the Fermi level to move downwards, giving a lower dark conductivity. Thermal annealing helps the potential barrier to be overcome, assisting a gradual return to the original T_3^0 states. (An inverse effect occurs when the initial position of the Fermi level favours the conversion of neutral acceptors to donors.)

Although the Staebler-Wronski effect is of little importance for the operation of solar cells (because the photoconductivity changes are too small to have much effect on the cell resistance), it does demonstrate the complexity of the defects in this disordered solid. These must be better understood before it can be told whether their presence is unavoidable. And even as things are, useful solar cells can be made from plasma-deposited amorphous silicon of existing quality, while other devices are not far behind. Further improvements in the material, either during production or by treatment afterwards, should still be possible. $\square$

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Corrigendum from B.T. Pickering

In commenting in these columns on the sequence of the common precursor to vasopressin and its neurophysin I stated that it held no surprises (*Nature* **295**, 280; 1982). However I also stated that the signal sequence is separated from that of vasopressin by a pair of basic residues which re-examination of Fig. 4 in the paper by Land *et al.* (*Nature* **295**, 299; 1982) shows to be incorrect. Indeed, had it been so, this would have been a surprise, since the membrane-associated cleavage of prepeptides is not thought to involve basic residues.

TEXTBOOK SUPPLEMENT

What makes a good textbook?

The most warmly welcomed textbooks are new editions of standard works.

What lessons should publishers draw?

THAT there is unlikely to be an immediate shortage of new textbooks is well attested by the pages that follow. In spite of the way in which most university systems are at present short of funds, publishers are still prepared to launch new products on student bookshops, while teachers of all kinds appear to have the time to put them together. On the face of things, at least one part of the academic industry is in good shape. Moreover, on the showing of this year's crop of books, there is no reason to suppose that the creationists' complaints about the compilation of textbooks for high schools in the United States, or even the hidden threat engendered by the recent trial in Arkansas, have their counterpart in higher education. Textbooks remain what they have always been — honest attempts to put between two covers (or, sometimes, four) an account of some field of knowledge and, frequently, some means of helping students to be sure that they have come to grips with it. Moreover, some of the fashions of the past few years seem now to be on the decline. The programmed-learning book, for example, seems far less common than a decade ago, partly no doubt because of the high cost usually involved but chiefly because students have apparently voted with their pocket books, and have thus told publishers that they do not like to be so tightly constrained. It is good to have this demonstration that the market functions.

So does it follow that the present offering of textbooks is precisely what the community of students needs? Unfortunately not. Even when it is accepted (as it should be) that students' reading is not confined to the textbooks on their recommended reading lists, even a cursory reading of the reviews that follow will show that the textbooks now available are not uniformly satisfactory, even by the objectives stated by the authors in the introduction. Why should this be? And how does it come about that the three new books on which reviewers have lavished something akin to affection are in their different ways idiosyncratic books? J.Z. Young's *The Life of Vertebrates* is now a classic, read by people other than students, and has managed to survive more than a decade out of print. Dirac's *The Principles of Quantum Mechanics*, now put into paperback in its fourth edition, has exactly the same status in a very different field and is as much an historical document as a textbook. (Indeed, students should be warned not to trust their conclusions if their arguments are as economical as Dirac's.) Sir Arthur Holmes's *Principles of Physical Geology* (long out of print) was, in its time, in the same mould — it was a robust and almost personal statement of the structure of the Earth which did not, for example, shrink from remarks about the plausibility of continental drift. Textbook writers (and their publishers) should prudently consider why these three volumes, and others like them, have been such great successes, intellectual and no doubt commercial.

The common properties of such volumes are easily listed. First, their authors were practising and enthusiastic teachers. (Though when Dirac's book first appeared, more than half a century ago, he was by no means an experienced teacher.) Second, the enthusiasm has been transferred from the classroom to the page. Third, perhaps as a consequence, the selection of topics and the distribution of emphasis is not what would be recommended by a solemn academic committee brooding about the exact balance of some students' course. Fourth, the sweep of all these books is

grand; the reader is left in no doubt that the essence of the field concerned has been fully described, even if a great many details have been omitted, which is most probably why they have all appeared in one edition after another. Finally, the language in which the text is written has an interest of its own. These three books are more than merely literate. Each also has a distinctive and even memorable style that somehow impresses on the reader's mind a sense that he is acquiring not merely information but an imaginative assessment of it.

For publishers, these reflections are no doubt discouraging. If textbooks of distinction require of their authors such a blend of uncommon qualities, the chances of outstanding success must be very small. Fortunately, the prospects are not so gloomy. Often, a group of people may be found collectively to show the qualities of a single talented author. One interesting example among this year's textbooks is *The Principles of Neural Science*, edited by E.R. Kandel and J.L. Schwartz but in reality an apparently accurate reflection of the interests and enthusiasms of the strong research group at Columbia University. As the reviewer remarks, readers may learn less about the chemistry of the central nervous system than (at this level) they need to know — and they may also learn more about the motor system than they want to know. But the book, bulky though it is, makes good reading and has a character of its own.

The consequences, for the textbook trade, are straightforward. The principles that apply elsewhere in book publishing are still applicable. The first test that should be satisfied by a textbook proposal is that it should be organized around a clear intellectual objective — and not too much encumbered by extraneous chapters whose function is chiefly to satisfy course requirements in this or that department. Self-conscious attempts to define the content of textbooks by means of curriculum development, which have been valuable and successful in the schools, have not so far been of great value in higher education. The chief but obvious difficulty is that even undergraduate courses are these days bound to be evolving rapidly, while the committees that superintend curriculum projects are bound to dilute the vision of their most imaginative members. By the same test, there are also limits to what can be done, in advance of publication, to ensure that textbooks for use in higher education can be tested by representative groups of the intended users. For the outstanding textbooks are those which, by being available, influence the pattern of what is taught. As elsewhere in the book trade, publishers cannot hope to know in advance whether they have published an outstanding book or a mere potboiler.

Thus the charitable view of the continuing spate of textbooks is that it represents the efforts of publishers and their authors to produce books that will endure, influencing the pattern of teaching in the process. There is, unfortunately, more to be said. The numbers of apparently similar titles, especially in fields in which the scale of teaching is quickly growing, are too great to be explained simply by people's earnest wish to put a potentially worthwhile book to the test of time and the student market. Too often, new textbooks uncannily resemble well-established works. Too often, they reflect the narrow idiosyncracies of individual teachers or departments. And, too often, they leave their student users with the false impression that science is, after all, a dull business.

In Dirac's shadow

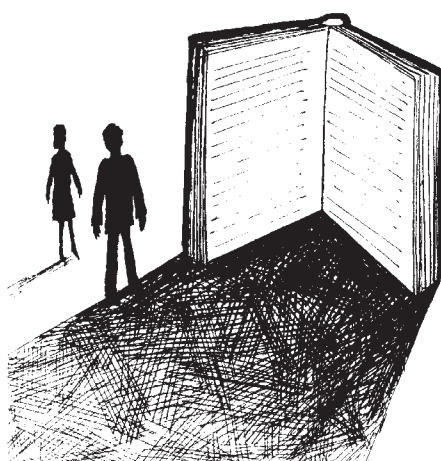
R.B. Jones

It is a malicious fate that sends a new book on quantum mechanics to be reviewed beside that of Dirac. It places an inhuman burden of restraint on the reviewer, whose first thoughts of Decline and Fall seem to require the pen of Gibbon or perhaps rather Evelyn Waugh. Dirac's classic textbook, *The Principles of Quantum Mechanics*, first appeared in 1930 but it remains incomparably the best book on the fundamentals of quantum theory. Here it is freshly reprinted as a paperback from its hardbound fourth edition, incorporating the revisions of 1967 on quantum field theory.

For those not yet familiar with it, let me say briefly that starting from the clearest of all accounts of the linear structure of quantum mechanics it proceeds to cover all the standard non-relativistic aspects of quantum mechanics other than variational techniques. In addition, the relativistic single electron and the quantum field theory of photons, electrons and positrons are described with masterly clarity and economy. Only the section on field theory may be regarded as provisional or seriously incomplete, since there is no hint of the rich development of quantum field theories since 1967 for the description of leptons and hadrons. All serious students should read this book but it demands unflagging intellectual courage. Moreover, it seems to get better and to yield more insights with increasing experience on the part of the reader. For these reasons almost no lecturer dares to use it as a standard textbook.

It is doubtless for similar reasons that we have, year after year, yet another crop of new books on quantum mechanics for irascible reviewers to condemn. John Martin's *Basic Quantum Mechanics* is meant as an introductory book for physicists. His approach is modern and non-historical, and from the beginning he banishes classical thinking in favour of the calculus of probability amplitudes. This approach is shown to good effect in his use of the Stern-Gerlach experiment to set up the two-component description of electron spin. He treats the standard solvable problems and then covers time-independent and time-dependent perturbation theory through the golden rule and the resonance approximation. There is a short account of dipole radiation and finally an introduction to the variational principle. Many familiar topics, however, are missing. There is very little discussion of the uncertainty principle

The Principles of Quantum Mechanics, 4th Edn. By P.A.M. Dirac. Pp.314. Pbk ISBN 0-19-852011-5. (Oxford University Press: 1981.) £7.95, \$29.50. *Basic Quantum Mechanics*. By J.L. Martin. Pp.241. Hbk ISBN 0-19-851815-3; pbk ISBN 0-19-851816-1. (Oxford University Press: 1981.) Hbk £17.50, \$39.50; pbk £7.95, \$17.95. *Quantum Mechanics*. By Hendrik E. Hameka. Pp.387. ISBN 0-471-09223-1. (Wiley: 1981.) £24, \$43.25. *Quantum Mechanics For Applied Physics and Engineering*. By Albert Thomas Fromhold, Jr. Pp.430. ISBN 0-12-26915-4. (Academic: 1981.) £22.60, \$34.



and no mention of the geometrical shape of wave functions or of the link between nodes and quantum numbers. There is no commutation rule treatment of orbital angular momentum eigenvalues, no three-dimensional scattering, no exclusion principle. In spite of many such omissions, this is a good, well-written introductory book with a distinctive point of view to justify its existence.

Quantum Mechanics by Hendrik F. Hameka is a book written for chemists. His approach is historical and primarily wave mechanical; he has made the book self-contained in its mathematical techniques to a degree not necessary for physics students but perhaps helpful to chemists. In this respect he over-emphasizes the role of special functions at the expense of matrix mechanics aspects of the subject. His treatment of the standard solvable examples is undistinctive and for me the book only came to life with the treatment of approximation methods. There is a nice combination of time-independent perturbation theory with variational techniques to include perturbations about

approximate eigenfunctions. Time-dependent perturbation theory includes an introduction to line widths and lifetimes. There is both a semi-classical and a second quantized treatment of atomic radiation. Spin and the exclusion principles are discussed, with a clear introduction to Slater determinants and Slater orbitals in the Hartree-Fock treatment of many-electron atoms. There is very little on angular momentum addition and no three-dimensional scattering theory. This is a reasonable textbook for a course biased towards atomic structure but, while perhaps an attractive book for chemistry students, it will not be a text of first choice for physicists.

Albert Thomas Fromhold, Jr, calls his book *Quantum Mechanics for Applied Physics and Engineering* and intends it as a two-year course for technologists working with solid state devices such as tunnel diodes or Josephson junctions. It is, in fact, three books in one for he covers elementary quantum mechanics, statistical mechanics of ideal quantum gases and solid state physics — an ambitious venture which does not succeed to my mind because these three subjects appear in unbalanced and piecemeal form. Thus in the section on quantum mechanics the simple harmonic oscillator and the hydrogen atom are omitted, apart from quoting their energy levels, while endless pages are devoted to one-dimensional tunnelling probabilities for square and trapezoidal barriers in WKB approximation. One learns about the almost free electron model of metals but nothing serious about phonons or magnetic properties of solids. The book is well produced and copiously supplied with problems and illustrations, but I do not think this approach will produce broadly based or coherent understanding. It would not take so much longer to learn these subjects as a physicist would by using the excellent textbooks already available and, incidentally, obtaining a much broader understanding.

One last stone remains to be hurled at each of the three books just considered. The authors treat mathematics as a subject which will terrify or confound their readers. It is in fact the natural language of quantum mechanics, and should be used without comment rather than segregated into separate sections marked "Dangerous". □

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Variations on cosmology and astrophysics

Joseph Silk

The Physics–Astronomy Frontier. By Fred Hoyle and Jayant Narlikar. Pp.483. ISBN 0-7167-1160-5. (W.H. Freeman: 1981.) £16.60, \$23.95. *Relativistic Cosmology: An Introduction.* By Jean Heidmann. Pp.168. ISBN 0-387-10138-1. (Springer-Verlag: 1980.) DM48, \$28.30. *Cosmology*, 2nd Edn. By Michael Rowan-Robinson. Pp.152. Hbk ISBN 0-19-851857-9; pbk ISBN 0-19-851858-9. (Oxford University Press/Clarendon: 1981.) Hbk £12.50, \$44.50; pbk £5.95, \$17.95.

IN ANY rapidly developing area of science, the textbook writer should do more than prepare a guide, no matter how thorough, to past accomplishments in his field. He must capture some of the excitement of developments in research, of new and tentative discoveries, of competing claims and counterclaims. An up-to-date treatment is especially crucial in the field of astrophysics, where the student is confronted in the daily press with a barrage of half-digested reports of new advances.

The texts by Jean Heidmann and by Fred Hoyle and Jayant Narlikar are failures by this measure. This defect is unfortunate since both books possess refreshingly novel features. Heidmann's text is a translation from French, originally published in 1973. Few revisions were made for the English edition, affecting only about five per cent of the text. The field of cosmology has developed enormously in the past decade; little of this is reflected in *Relativistic Cosmology*. However an unusual feature is the attempt, on the whole successful, to make esoteric topics in differential geometry accessible to the non-specialist.

The book begins with a discussion of topics in extragalactic astronomy that are of interest to the aspiring cosmologist, including clustering of galaxies, the expansion of the Universe, and intergalactic matter. The text, though, suffers from a lack of modern results. Although the intent is for the reader to fill in details by reference to the bibliographies, this will be difficult, since the references, almost without exception, stop at 1978. The section on the differential geometry of curved spaces is lucid and interesting. My only quibble is that it is almost irrelevant to the rest of the book. The remaining sections consist of a discussion of the Friedmann–Lemaître cosmological models and of rudimentary observational cosmology. While the level of this book is appropriate for a first-year course for an undergraduate with a mathematics and physics background, it is difficult to imagine any course being based on this text without an enormous amount of supplementary material.

By way of contrast, *The Physics–Astronomy Frontier* does provide a self-contained account of astrophysics. Its aim

is to describe astronomy from the perspective of a physicist. Consequently, each section begins with a discussion of one of the fundamental forces of nature (electromagnetic, nuclear weak and strong, and gravitational) before moving on to the astronomical phenomena. This book is especially interesting on historical developments and anecdotes — such as when Hoyle reminisces about how Walter Baade almost discovered the pulsar in the Crab nebula — and the early years of radio and X-ray astronomy are well covered. However, the authors do not mention any observational discoveries of the past five years. Consequently, the explosive growth in radio and X-ray astronomy and other fields is ignored. This is especially unfortunate in a book that purports to be probing the frontiers of astronomy and physics. One notable omission is any discussion of particle physics in the very early stages of the big bang. Instead, the reader is offered an exotic cosmological theory, developed, and possibly only taken seriously, by the authors themselves.

The Physics–Astronomy Frontier is written for students who possess a strong secondary school science background. The text is mostly non-mathematical. Does it succeed, as the authors hope, by focusing

on the fundamental forces of nature in enabling the reader to actually acquire a physical understanding of astronomical phenomena? Certainly it is far superior in this regard to other astronomy texts at a comparable level. However, the astronomical contents are deficient and dated; at best, one might use the Hoyle–Narlikar book to complement a more conventional astronomy text assigned for an undergraduate course for non-specialists.

Michael Rowan-Robinson's second edition of *Cosmology* is an updated version of a text originally published in 1975 for first-year mathematics and physics undergraduates. It provides a self-contained description of the visible Universe, including both galactic and extragalactic phenomena of interest to the observational cosmologist. There are minor omissions, such as any discussion of chemical evolution, but a reasonably thorough basis is established for cosmological theory. The meat of the book lies in its discussion of the big bang models and of their observational implications. This is a well-written summary of cosmology, which aspires (not without good reason) to be a successor to Hermann Bondi's text of the same title that had such a profound influence on undergraduates two or three decades ago. □

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Life of cosmic rays

G.T. Bath

High Energy Astrophysics. By M.S. Longair. Pp.412. Hbk ISBN 0-521-23513-8; pbk ISBN 0-521-28013-3. (Cambridge University Press: 1982.) Hbk £24, \$49.95; pbk £8.95, \$19.95.

IT IS a considerable task of synthesis to show the connections between the main fields of astronomy which have flourished in recent years and at the same time convey the drama of research at the frontiers. It must range from the solar wind to clusters of galaxies, from radiation processes in the presence of strong magnetic fields to the space–time structure of black holes. This is the ambitious aim of this introductory textbook — to show how astronomers work, how they think and how the subject has developed — by introducing the reader to many of the discoveries of the past 20 years, and by explaining some of the theoretical interpretation that has survived the rigours of criticism and discussion.

But this book is not simply an exposition of modern astrophysics. The author adopts a quite original approach. Taking as his central theme the subject of cosmic rays, their detection and the physics of their interaction with the environment, the

presentation derives the necessary basic physics to trace the cosmic ray propagation path through the atmosphere, the magnetosphere, the solar wind, and the interstellar and intergalactic medium, back to the production of high energy particles in galactic and extra-galactic objects. The high energy astrophysics of the title refers to these ultimate sources of cosmic rays in the Universe, which are introduced as their peregrinations through the hazards of space are traced back to their birth.

The author makes no apology for presenting the material, and referring to it, as a lecture course. Indeed his personality and enthusiasm shine through the informal and stimulating style of the writing. The depth of discussion varies from subject to subject. In a book which introduces cosmic ray physics and detectors, basic astronomy at all wavelengths, stellar evolution and stellar death, the properties of galaxies and clusters of galaxies, and high energy astrophysics, this is not surprising. As a stimulating undergraduate introduction to particle propagation at high energies, and the astrophysical environment in which it all takes place, this book gives a broad background without losing contact with the dynamism that created the subject.

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Blinded with theory

J.S.A. Green

An Introduction to Atmospheric Physics, 2nd Edn. By Robert G. Fleagle and Joost A. Businger. Pp.432. ISBN 0-12-260355-9. (Academic: 1980.) \$29.50, £19.60. *An Introduction to Atmospheric Radiation*. By Kuo-Nan Liou. Pp.392. ISBN 0-12-451450-2. (Academic: 1980.) \$34.50, £22.80.

WE LIVE in a world where physics students seem to believe that if a process can be visualized, it must be trivial. Thus the really important things to study are theoretical physics, quantum mechanics and nuclear physics. A subject such as biophysics becomes more respectable if the object of study is first reduced to a molecular soup, less so if an actual tangible biological entity is involved. Meteorology comes somewhere in the middle because, though we have our share of specialized incommunicable topics, the essential feature of the subject is the bringing together of many branches of physics in a way that can lead to understanding the very tangible phenomena presented by the weather.

Fleagle and Businger cover aspects of cloud microphysics, scattering and absorption of electromagnetic radiation, fluid dynamics and photochemistry at a level acceptable to a graduate student of physics or mathematics. The text is clear, but as in any such collection of work from different specializations notation is a problem and the authors' suffix notation gets out of hand at times. Worked problems are used to extend the students' appreciation, though it takes a lot of will power not to cheat and look at the solution straight away. Definite improvements to the 1963 edition are the addition of a useful chapter on dynamics and more modern theory of turbulence, with the rather isolated chapter on geomagnetic effects now being omitted.

Liou deals with the transfer and detection of solar and terrestrial radiation, aimed perhaps at the student anxious to study data provided by the earth-satellite programme. Recommendation is difficult because the book is so carelessly written. The text is distinctly oriental at times — cgs and calories are dominant units, rainbows are a consequence of scattering and $1\text{cm} = 30\text{ GHz}$. A more conventional explanation of the rainbow is given later and the chapters on Mie theory and multiple scattering are extensive.

Something, however, is missing from both books and finally I concluded that it was that there isn't actually any meteorology in them. Never do the authors dig you in the ribs and mutter conspiratorially "there — you have seen that phenomenon before, and never realized the beauty of the physics behind it". Thus both books suffer because they do not make use of the students' (or the

authors') prior knowledge of what the atmosphere is like. For judgement, context and excitement the student would too often have to refer to the original texts; and so, he might as well study quantum mechanics.

Most telling to me is that the authors do not use their eyes. Liou's three-dimensional diagrams (pp.130 and 222, for example)

look as though they were drawn by Escher for purposes other than conveying information about physics. Fleagle and Businger's are not much better. □

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Mixed diet for students of climatology

J.P. Palutikof & T.M.L. Wigley

An Introduction to Climate, 5th Edn. By Glenn T. Trewartha and Lyle H. Horn. Pp.416. ISBN 0-07-065152-3. (McGraw-Hill: 1980.) £18.25, \$32.95. *Climatology: Selected Applications*. By John E. Oliver. Pp.260. ISBN 0-7131-6303-8. (Edward Arnold/Halsted: 1981.) £14.95, \$34.95. *Mountain Weather and Climate*. By Roger G. Barry. Pp.313. ISBN 0-416-73730-7. (Methuen: 1981.) £17.50, \$33.25.

If climatology were breakfast, these three books would be eggs Benedict (Barry), Spanish omelette (Oliver) and rather old porridge (Trewartha and Horn). Barry's book is imaginative and nourishing, but perhaps a little esoteric for everyday tastes; Oliver's, original in concept although not totally successful in execution, is nevertheless full of good things. As for Trewartha and Horn, while the book still has some nutritional value, the average breakfast eater would probably choose to eat out.

An Introduction to Climate is the fifth edition of a book which was first published in 1937. The style is still reminiscent of the pre-War approach to climatology, although in this edition some effort has been made to give the book a "more modern cast". Unfortunately it needs a completely new script as well as a new cast. Much of the book is devoted to the Köppen classification of climates, and little attention is paid to modern developments in climatology. One hopes that few lecturers or teachers today would structure an introductory course on climate with this sort of emphasis. As an illustration of the old-fashioned approach, the data given in the form of maps, diagrams and tables rarely have any indication of the period represented, the reliability or the source. This denies two key issues in modern climatology — the facts that climate *does* change, on all time-scales, and that our knowledge of *global* climate is very meagre. The book is at best misleading, at worst wrong; it provides a salutary lesson that a time must come when updating (even if it is done well, which it is not here) is no longer a useful exercise.

The stated aim of *Climatology: Selected Applications* is to "supply appropriate real-world applications" for basic university courses in climatology. The

author takes in turn each of the themes which form the content of such courses, summarizes it, and then proceeds to provide case studies of the incidence and impact of the climatological features involved. To take one example, Chapter 7 begins with a general description of global circulation patterns, then examines in detail the synoptic patterns of the severe winter of 1976–1977 in the United States and finally considers the impact of this unusual climatic event. This is followed by a complementary analysis of the contrasting summers of 1975 (wet) and 1976 (dry) in the US midwest. The approach is a new and valuable one, which for many teachers will supply a long-felt need. For others, awareness of this text may well provide a new dimension to teaching.

The book is likely, therefore, to be widely read and have considerable influence. As a first attempt, it must be praised. Criticism may be levelled at it for being somewhat shallow, although this can be rectified in subsequent editions when the concept is better established. British readers will perhaps think that the concentration on examples from the United States is disappointing; others will find the system of blocking the data analysis sections into separate boxes disrupting to the flow of the text. Perhaps the greatest compliment one can give any textbook is that it makes learning interesting and, dare we say it, fun. In this, Oliver's book certainly succeeds.

Barry's *Mountain Weather and Climate* is a unique volume on a special subject. He discusses all aspects of the weather and



climates of mountainous regions. The coverage is global and the framework very general, but the material presented is invariably most detailed and specific. As an example of a specialist work it is excellent:

ideal as the basis for an upper-level undergraduate or graduate course, and sufficiently detailed and authoritative that it can act as a comprehensive and up-to-date reference work. Since no other publication covers this field, and because the author perceives well the far-reaching implications of the subject matter, it succeeds also as a basic source book for botanists, foresters, glaciologists and hydrologists. The bibliography is most impressive, but unfortunately there is no author index. This is a mere token criticism, however. Although a specialist work, this book should be on the shelf of all climatologists and all those interested in the mountain environment.

Comparing three such different books is an invidious task, one which we shall not attempt. The fact that, in a "small" discipline like climatology, such diversity is possible illustrates the mushrooming growth of the subject in recent years. The different approaches of Trewartha and Horn on the one hand and Oliver on the other are an indication of the extent to which the subject has developed. Both Oliver's and Barry's books show how much climatology impinges on many other disciplines, and are harbingers of future developments. □

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Earthly delights . . .

Keith Bell

Essentials of Earth History: An Introduction to Historical Geology, 4th Edn. By W. Lee Stokes. Pp.577. ISBN 0-13-285890-8. (Prentice-Hall: 1982.) \$25.95, £19.45. *Evolution of the Earth*, 3rd Edn. By Robert H. Dott and Roger L. Batten. Pp.573. ISBN 0-07-017625-6. (McGraw-Hill: 1981.) \$31.50, £17.50.

TO WRITE a well-balanced, comprehensive textbook at the introductory level about the history of the Earth, palatable to both arts and science students alike, offers something of a challenge, particularly at the present time. Results of space exploration, as well as new findings in plate tectonics and evolution (dare I mention the word?) must be fitted into one compact volume along with at least some of the basic principles of stratigraphy.

Exciting introductory textbooks are rare. My own favourite used to be the second edition of Arthur Holmes's *Principles of Physical Geology* (Ronald Press, 1965), a book written in a bold, masterly style, brimful of ideas and still retaining a freshness some 20 years later. But what of present-day texts?

Under review are new editions of two

books that have been popular in North American universities for some years. Both are well-written, profusely illustrated, accurate and surprisingly similar both in scope and in content. Diverse topics such as the evolution of the Solar System, the origin of life and palaeomagnetism are included, along with a generous serving of historical geology. Both books retain layouts similar to the earlier editions. Stokes's text, however, has been extensively rewritten and many new illustrations either supplement or replace those of the previous work.

Stokes's approach is the more cautious and circumspect of the two. Although *Essentials of Earth History* tends to be written in a dry, somewhat formal style, the book is by no means dull. The well-illustrated volume contains a number of excellent photographs, including some from the recent Pioneer and Viking missions. Most chapters are self-contained (Chapter 7, "Cosmic Beginnings", was particularly enjoyable). And, under the heading "Search for an Ethic" in the final chapter, it was interesting to see at least a token attempt to reconcile present evolutionary theory with religious beliefs. Stokes, throughout most of the book, seems to have the knack of anticipating students' questions and needs; rather than evading contentious issues, the author confronts most of them head on.

The book by Dott and Batten is generally more direct and the reader is led at a fairly brisk pace from start to finish. The diagrams are superb and are among the clearest that can be seen in any introductory textbook. Topics such as geochronology, plate tectonics and the early evolution of the Earth are handled with a minimum of jargon and in a straightforward, concise style. However, I suspect that those chapters dealing with historical geology, mainly of North America, may overwhelm most students; each of them contains too much detail and too many disjointed topics. Although the introduction of many concepts within each chapter was probably meant to challenge, the overall effect may be to daunt all but the most enthusiastic students. Chapter 14, for example, includes discussions about sedimentary cycles, the red-bed problem, the Appalachian Orogeny, the significance of thrust-faulting and Palaeozoic land floras!

Nevertheless, either book would make a good introductory text, though less stratigraphy and a little more about igneous and metamorphic rocks (mentioned but briefly) would certainly extend the scope of both of them. An interesting feature of each book is a glossary of geological terms — to any student just coming into the earth sciences this is particularly useful. □

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. . . heavenly bodies

L. Wilson

Earthlike Planets: Surfaces of Mercury, Venus, Earth, Moon, Mars. By Bruce Murray *et al.* Pp.387. Hbk ISBN 0-7167-1148-6; pbk ISBN 0-7167-1149-4. (W.H. Freeman: 1981.) Hbk \$27.50, £20.40; pbk \$15.95, £10.95.

THE constant development of the planetary sciences presents problems for the writers of textbooks, since such works are in real danger of being significantly out of date, at least in parts, by the time they are published. This problem is avoided by the authors of *Earthlike Planets* by omitting all mention of the gas giants, Jupiter and Saturn, and their satellites (targets of the most recent planetary probe missions), and confining attention to the more truly Earth-like inner planets — Mercury, Venus, the Moon and Mars. Many aspects of the major stages of evolution of these bodies are now well understood and it is becoming possible to make useful comparisons between these planets and the Earth, both in terms of the histories of their interiors and of the geological, physical and chemical processes which have moulded their surfaces.

Indeed, this book is explicitly committed to the concept of comparative planetology and the first four chapters deal with various aspects of the study of all the inner planets taken as a group: a summary of changing ideas on the ways in which these bodies were formed; a global description of the surfaces and atmospheres; a review of processes modifying planetary surfaces (impact by meteoroids and asteroids, mass movement under gravity and, where appropriate, wind erosion and fluvial action); and a survey of the volcanic and tectonic processes which renew planetary crusts. The next three chapters are explicit attempts to synthesize the planetary history for each of the Moon, Mercury and Mars. Finally, emphasis returns again to comparisons, with a review of those processes which appear to have influenced all of the inner planets to varying degrees — impact modification of the earliest-forming crust, magmatic and tectonic evolution of the interior and, on the planets with atmospheres, climatic change.

Each chapter ends with a well-chosen list of books and papers for further reading, and an excellent guide to sources of planetary photography is included as an appendix before the index. *Earthlike Planets* is probably the best general introduction to planetology available at the moment and will find a place in undergraduate courses at all levels. It is equally suitable as a starting point for a postgraduate student entering the planetary science field. □

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Tectonics as theory, tectonics as history

B.C. Burchfiel

Geology of the Continental Margins. By G. Boillot. Pp.115. ISBN 0-582-30036-3. (Longman: 1981.) £4.95, \$11.95.
Geotectonics. By V.V. Belousov. Pp.330. ISBN 0-387-09173-4. (Springer-Verlag: 1980.) DM48, \$28.40. *Tectonics and Landforms.* By Cliff Ollier. Pp.324. Hbk ISBN 0-582-30032-0; pbk ISBN 0-582-30033-9. (Longman: 1981.) Hbk £20, \$55; pbk £9.95, \$24.

WITH our rapidly increasing understanding of global tectonics, books published on the subject are commonly outdated within a few years of publication. Two of the books reviewed here (*Geology of Continental Margins* and *Geotectonics*) are translations of previously published works, which originally appeared several years ago and



contain material which is already somewhat out of date. *Tectonics and Landforms*, in contrast, promises an interesting approach and in some instances makes excellent use of recent tectonic concepts and their relationship to geomorphology. However, books on such broad topics often cannot adequately cover each subject within their scope, and this one is no exception.

Geology of Continental Margins is a translation of a book first published in French in 1978. No subject in the earth sciences has evolved more rapidly in the past five years, which renders the book introductory at best and outdated at worst. While some of the material presented is still generally valid, recently developed details of continental margin evolution, particularly passive margin evolution, are obviously missing.

Instead of focusing on the great wealth of information which now exists about the geology of the continental margins of the world, the book concentrates on plate tectonic models of continental margins and presents little real data; if readers are looking for factual information on particular continental margins, they won't find it in this book. I was also surprised to find lengthy discussions of subduction, the deep seismic structure of subduction zones,

paired metamorphic belts, geosynclines and collisional tectonics.

Although advertisements for the book suggest that some updating has been done for the English edition, it seems to have been minor. Some articles published in 1979 have been added to the "further reading" section, but little new data appears in the written part of the text. In my view this is a model-orientated, highly simplified presentation of continental margins. The emphasis is on the plate tectonic development of continental margins, rather than on what they are really like.

Geotectonics is a slightly-updated translation of a book published in Russian in 1976. Some of the data presented were published as recently as 1978. Even so, the book presents a view of the geotectonics of the Earth in the well-known and unique conceptual framework developed by Belousov himself. In the preface it is suggested that the reader should be familiar with the principles of tectonophysics developed in Belousov's *Structural Geology* (published in 1978, in French). I think this would be advisable, as much of the terminology and some of the conceptual ideas in this work need such a background.

Most of the geological examples and figures presented are either from older works — 1960s or earlier — or are Belousov's reinterpretations of work by other scientists. The examples cited, as well as those not presented for the same area, were apparently selected to develop the author's particular view of geotectonics. Vertical movement of material in the crust, mantle and core remain the dominant principle of Belousov's view of the evolution of the Earth; horizontal movements of rocks are considered to be of limited importance. Basification of continental crust leads to the formation of ocean crust. These and other concepts developed by the author are generally not acceptable to many of us, but deserve publication. If we cannot explain more adequately the geotectonic evolution of the Earth, and do not continue to test all reasonable hypotheses, earth science will stagnate.

Except for a five-page discussion of plate tectonics near the end of the book and another seven pages of critique, the book is all Belousovian tectonics and will be of interest to only avid scientists interested in the subject.

The purpose of *Tectonics and Landforms* is to relate tectonics and structure to landforms on a scale which ranges from continents and oceans to local surficial features. This is truly an admirable purpose and potentially very exciting and interesting. With such a broad purpose but abbreviated length, the presentation has some successes, but inevitably some

subjects are treated superficially. Much of Ollier's presentation focuses on large-scale tectonics, developing the concepts of plate tectonics and its resultant geomorphic features. From this basis, a great variety of subjects, such as flow of ice and rock, planation surfaces and drainage patterns are covered in very short sections. Not only are modern concepts developed in the book, but reviews of older, historical, and in many cases obsolete, theories are also presented.

I was particularly pleased to see that many of the pioneering ideas on tectonics by S.W. Carey are discussed, but I doubt whether a chapter on the expanding Earth was necessary, particularly when most of the book is devoted to the development of plate tectonics. In several places the author gives plate tectonic explanations for various features followed by a disclaimer that other explanations which are incompatible with plate tectonics, such as interpretations based on expanding Earth models, should not be discounted.

Tectonics and Landforms is well written, well organized and well illustrated. Although the author claims to aim at senior undergraduates, in my opinion the book is too superficial for students at that level. Rather, it would be better suited to an introductory audience, but even then offers a rather uneven overview of a great variety of earth science subjects. □

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Crystal growth

R.A. Howie

Crystallography: An Introduction for Earth Science (and Other Solid State) Students. By E.J.W. Whittaker. Pp.254. Hbk ISBN 0-08-023805-X; pbk ISBN 0-08-023804-1. (Pergamon: 1981.) Hbk £13.50, \$32.50; pbk £8.35, \$19.95. *Mineralogy for Students*, 2nd Edn. By M.H. Battey. Pp.355. ISBN 0-582-44005-X. (Longman: 1981.) £8.95, \$19.95.

Crystallography is presented in two evenly-divided parts: the first deals with morphological crystallography, symmetry and systems, stereographic projections, and derivation of symmetry and forms in all 32 crystal classes, while Part II covers the fundamental features of X-ray crystallography, the 14 Bravais lattices, the interpretation of powder photographs, X-ray diffraction by single crystals and symmetry in repeating patterns. Part I thus somewhat overlaps in coverage with *An Introduction to Crystallography* (4th Edn, Longman; 1971) by F.C. Phillips and A.C. Bishop's *An Outline of Crystal Morphology* (Hutchinson, 1967) though,

like the former, it aims to be of use not only to geologists and mineralogists but to other students of the solid state.

Although both of these older texts (and *Mineralogy for Students*) give relatively brief treatment to X-ray crystallography, Part II of Whittaker's book covers this expanding field in considerable detail and develops a number of novel features in response to the difficulties and attitudes of students who are unlikely to have a primary interest in morphological crystallography. There are brief but useful chapters on electron diffraction, the determination of crystal structures and on irregularities in crystals (though here twinning is touched on rather too briefly: whether or not we understand why and how twins form they are frequently to be seen under the microscope and may be of considerable relevance). The book concludes with a brief essay on the relationship between morphology and crystal structure. Altogether this elegant text should be a worthy successor to that of F.C. Phillips. As Whittaker states in his introduction, mineralogy is merely the first branch of solid state science to be developed, and structural crystallography is equally relevant to all students of the solid state.

The format and most of the text of the new edition of *Mineralogy for Students* are identical with that of the first. The only fresh feature is the inclusion of a new series of optical orientation diagrams for most of the common rock-forming minerals, but whether this justifies the whole work being labelled as a second edition is, in my opinion, doubtful. Nevertheless this was a useful, well-written text when it first appeared in 1972 and is improved by the additional diagrams — though it is a pity that these are not presented at the appropriate places in the section on descriptive mineralogy.

The book has some things in common with *Crystallography* in that it deals with the principles of crystal structure, crystal morphology and X-ray diffraction in some 85 pages, a reasonable proportion for an elementary mineralogy course. It has the advantage for students of including both the principles and methods in Part I (comprising just under half of the book) and descriptions of minerals in Part II, compared, for example, with the rather more detailed descriptive mineralogy (but without any descriptions of techniques) provided by Deer, Howie and Zussman's *An Introduction to the Rock-Forming Minerals* (Longman, 1966). Dr Battey's text is well illustrated and presented, though surely the formula of chloritoid could be given in a rather simpler fashion than one involving four consecutive brackets; the book will nevertheless continue to be deservedly popular with students. □

R.A. Howie is Professor of Mineralogy at King's College, University of London, and Principal Editor of Mineralogical Abstracts.

Mixed sediments

R.C. Selley

Sedimentary Petrology: An Introduction. By M.E. Tucker. Pp.252. ISBN 0-632-00074-0. (Blackwell Scientific/Halsted: 1981.) £8.50, \$24.95. *Sedimentology of Shale: Study Guide and Reference Source.* By Paul E. Potter, J. Barry Maynard and Wayne A. Pryor. Pp.303. ISBN 0-387-90430-1. (Springer-Verlag: 1980.) DM59.90, \$32.80. *Depositional Sedimentary Environments: With Reference to Terrigenous Clastics*, 2nd Edn. By H.-E. Reineck and I.B. Singh. Pp.549. ISBN 0-387-10189-6. (Springer-Verlag: 1981.) DM59, \$34.90.

THE three books considered in this review all deal with the study of sedimentary rocks but differ widely in subject area, level of assumed knowledge and approach.

Tucker's *Sedimentary Petrology* is an excellent textbook for second-year geology students. An introduction is followed by chapters dealing with sandstones, shales, carbonates, evaporites, ironstones, phosphates, coal, chert and volcanoclastic sediments, each concluding with a selective reading list. There are many clear line drawings and photographs, though some of the latter have suffered from over-reduction.

The chapter on sandstones (conglomerates and breccias) also briefly describes sedimentary structures and processes, the textural properties of grains and sediments, and the concept of facies. That on limestones (including dolomites) deals adequately with the complex genesis of the various carbonate grains and presents a synthesis of the horrors of carbonate diagenesis.

These two chapters make up nearly half of the volume and it is by them that the work as a whole will be largely judged. Basically they are excellent introductions which lay the foundation for later, more advanced study. Even the concept of porosity is introduced, and there are short sections on the porosity of sandstones and carbonates.

The book as a whole is most nearly comparable with Greensmith's *Petrology of the Sedimentary Rocks* (Thomas Murby, 1975). Both deal with sedimentary rocks largely from the microscopic as opposed to the megascopic aspects. Tucker's book, however, lacks a chapter on weathering so students may not be too clear on where on Earth all this sediment actually comes from.

Sedimentology of Shale is a very different book. Until recent years, shale had long played Cinderella to the ugly sisters of sandstone and limestone. The advent of modern analytical techniques, however, coupled with the quest for oil shales and oil source rocks, has stimulated interest in the mud rocks. The book is subtitled *Study Guide and Reference*

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Source. This must be noted for the work is unconventional in format. It contains but three chapters: "Overview", "Question Set" (*sic*) and "Annotated and Illustrated Bibliography". Chapter 1 covers the mineralogy and chemistry of clays, but ranges widely from Bouma sequences to ichnofacies. The second deals with methods for analysing shales, ranging from descriptions of the measurement of a section to the reflectance of phytoclasts. Fifteen colour photomicrographs illustrate various shale microfacies.

It is the third chapter that is unconventional. This takes up over half of the book and consists of an annotated and illustrated reference list. It is divided into sections with headings ranging from "Books, Classics and Classification" to "Shales as Hosts for Metallic Minerals" and "Environmental and Engineering Geology". Within each section the authors have arranged alphabetically references which they deem to be significant.

A sentence or, at the most, a paragraph explains the relevance of each reference, and key illustrations are reproduced from the original papers. This is a novel and

painless way of "writing" a book. There may be some customer resistance to buying a volume which is largely an annotated bibliography — many geologists may prefer to use *GeoAbstracts* or a computer-based literature retrieval system. For those who do not, *Sedimentology of Shale* will prove to be an essential source book; pity, though, that with three authors "desiccation" is misspelt throughout.

Of Reineck and Singh's *Depositional Sedimentary Environments* there is far less to say. The first edition appeared in 1973 and rapidly became established as the definitive work on recent terrigenous sedimentary processes, structures and environments. The standard of the second edition ensures that it will maintain this position. A major re-write has not been attempted, but the book has been updated throughout and sections have been added to each chapter on ancient environments. Nonetheless, the main emphasis of the book remains on recent terrigenous sediments. □

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From the abyss to the lecture theatre

Peter J. Smith

Marine Geology. By James P. Kennett. Pp.813. ISBN 0-13-556936-2. (Prentice-Hall: 1982.) \$34.95, £26.20.

NO DISRESPECT to Professor Kennett, but in one sense this huge volume is alarming. For only three decades ago hardly any of it could have been written. Only during the past 20 years or so has the vaguely peripheral topic of marine geology moved to centre stage as Marine Geology — and with such vengeance that it is now possible for Kennett to write a textbook of an astonishing 813 pages at the comparatively lowly undergraduate-early-graduate level.

What made the rise of marine geology possible was the development of the appropriate exploration techniques; but what has made it matter is the discovery of the oceanic lithosphere's central role in the plate tectonics story and the consequent realization that the ocean floor's evolution is altogether more complex and interesting than that of a passive sedimentary dumping ground of great antiquity. Holism is the keyword here; and despite the need to become expert in a bewildering variety of subdisciplines, Kennett is wise enough not to allow his presentation to disintegrate into its constituent elements.

Of course, the parts are important; and in a teaching text it is essential that they be dealt with in some detail. Thus well over half of the volume is devoted to the basics of ocean morphology, marine stratigraphy and correlation, the current plate tectonic framework, crustal structure, sea-level

changes, ocean margin processes, sediments and their microfossil loads, and so on. The extended climax of the book, however, is a long three-chapter section on palaeoceanography — a splendid synthesis of almost everything we know, or think we know, about how the ocean system has evolved.

This is the first major textbook on marine geology to appear since the plate tectonics revolution, although others are in progress in what could turn out to be a small publishing growth area during the 1980s. Because so much of the material is new and far from all of it has become part of the consensus, the book is necessarily referenced to an extent that undergraduates will find unusual, and perhaps even distracting, but graduates will find helpful. On the whole, Kennett has achieved a reasonable compromise between the need to attribute and the desire to find a lucid instructional style. The book is clearly intended chiefly for advanced undergraduates in the marine geology courses now springing up in the more forward-looking universities and colleges; but in view of the subject's crucial importance to all geology, it will prove indispensable to earth scientists at much higher levels and of all persuasions. Potential competitors will be hard pressed to improve on it. □

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Soils: their genesis and physics

David Rimmer & Michael Adey

The Soil Resource: Origin and Behavior. By Hans Jenny. Pp.377. ISBN 0-387-90453-X. (Springer-Verlag: 1981.) DM57, \$33.70. **Fundamentals of Soil Physics.** By Daniel Hillel. Pp.430. ISBN 0-12-348560-6. (Academic: 1980.) \$40, £26.40. **Applications of Soil Physics.** By Daniel Hillel. Pp.385. ISBN 0-12-348580-0. (Academic: 1980.) \$40, £26.40.

IT IS now 40 years since the publication of Hans Jenny's classic monograph *Factors of Soil Formation* (McGraw-Hill, 1941), a book which provided the framework for much subsequent work in soil genesis. Now in his eighties, Jenny has written what amounts to a sequel to that earlier work. In it he develops his ideas into a broader, ecological view, describing how, by analysis of the "state factors", either whole ecosystems or their separate components — vegetation, animals and soil — evolve with time and vary from place to place. This treatment occupies the second half of the book and is preceded by a consideration of the processes of soil genesis (water balance, ion exchange, clay formation, for example).

Jenny draws many of his examples from his own work and that of his students and colleagues. His use of the English language is unconventional; for example, he talks of "swarms" of ions around particles, "particle bondage with humus" and words like "porositors" and "depauperizes" appear. In addition the editors have purposely retained many of the original drawings which he made for the diagrams and figures, in order "to carry through the artistic touch". So what one gets overall is a very personal, even idiosyncratic, view of the soil resource. Although written in a textbook style, and in part intended for students, one would not recommend this as an undergraduate text. However the second part of the book particularly will be of interest to the many soil scientists who have been influenced, directly or indirectly, by the ideas of Hans Jenny.

In recent years a number of texts on soil physics have been published, rectifying the absence of much choice which had existed for some time. *Fundamentals of Soil Physics* together with its companion volume *Applications of Soil Physics* further extends the range of material in a most comprehensive way. Whilst each is a separate, self-contained volume, the latter does draw on the former and assumes an appreciation of the basic principles of soil physics. Together they cover the broad range of soil physics, providing a welcome integration of basic principles and practical applications.

Fundamentals . . . treats the basic static and dynamic properties of the solid, liquid

and gaseous phases in turn. After an introduction to the general physical characteristics of soils and basic properties of water, the solid phase of soil is studied through a consideration of particle size distributions and specific surface areas, the properties of clays, and structure and aggregation. The concept of soil water potential is carefully developed and used in a consideration of both saturated and unsaturated hydraulic conductivity and the movement of solutes. The roles of diffusion and respiratory activity in determining the composition of the soil atmosphere are considered and this leads naturally to a chapter on heat flow and temperature. Finally, soil strength, consistency and compaction are discussed.

The author introduces essential physics where appropriate and is thorough without being overly mathematical. Thus care has been taken to use both one- and three-dimensional forms of flow equations. Developments of theoretical aspects describing basic processes are complemented by discussion of both laboratory and field methods of measuring important parameters. Except, perhaps, for the lack of any coverage of the soil-plant-atmosphere continuum and erosion, this text alone should cover most undergraduate courses.

Applications . . . continues the discussion, paying particular attention to the management of the soil environment and the field-water cycle. Thus infiltration, drainage, irrigation, evaporation and soil moisture uptake by plants are considered, drawing on the basic principles introduced in the companion volume. These themes are elaborated in a second section consisting of five chapters by invited authors who cover further aspects of the field-water cycle and the problems of studying soil heterogeneity. These contributions dovetail well with the earlier section and give a useful insight into some of the more advanced realms of soil physics.

These two books provide a modern, clear and concise introduction to soil physics. They are international in style and content, drawing examples from a wide range of situations. Unfortunately the cgs system of units is used, though the metric equivalents are often given (however, the Pascal is not introduced when considering soil water potential). Whilst the texts do introduce an appreciable amount of mathematics, they should not prove too difficult for the careful student and understanding is aided by the excellent sample problems at the end of each chapter. Over 400 references (up to 1980) are given at the end of each volume, and provide a good starting point for further work.

Together, the two volumes represent an admirable textbook for undergraduates. They should certainly be in every library covering soil science. □

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Dirty work at the crossroads?

M.J. Kirkby

Soils and Landforms: An Integration of Geomorphology and Pedology. By A.J. Gerrard. Pp.219. Hbk ISBN 0-04-551048-2; pbk ISBN 0-04-551049-0. (George Allen & Unwin: 1981.) Hbk £15, \$35; pbk £7.95, \$16.95.

SOIL science and geomorphology have borrowed from one another throughout their joint histories and, as with other disciplines, the relationship has been very fruitful. Areas of mutual interaction have, however, largely relied on the static properties of soil or sediment, while understanding of process dynamics has followed rather divergent paths in the two disciplines. Pedologists have tended to concentrate on the chemistry, physics and biology of the soil, mainly at a micro-scale; while geomorphologists have attempted to understand sediment and solute transfers, generally at a macroscopic to world-wide scale.

One very important area of common ground has, I think, been surprisingly neglected. At its simplest, it springs from the obvious fact that contemporary soils are the very materials which are moved downhill by current landform processes. It follows that the physical and chemical processes by which landforms and soils evolve are substantially identical. To develop this theme — and there are signs that it is being increasingly developed — there is an urgent need to collate existing concepts and data; to translate concepts between the two disciplines; and to re-think many of our approaches in a new idiom.

John Gerrard's book makes a worthwhile contribution to the first of these three aims, by bringing together work on soil formation, on soil movement and removal, on hillside hydrology and on the catena concept which must be central to any integrative work. In reviewing this material there is however little evidence of fresh insights or of the author's own syntheses, so that little headway is made in translating concepts or adding to them.

About half of the book then proceeds to consider soils on the basis of the landforms on which they are formed, including soils on erosion surfaces, flood and coastal plains, and glacial deposits. In this section, there is the focus on landforms which might

be expected from the title, but not enough emphasis on the links with solution and other geomorphological processes. The final chapter, "A Pedogeomorphic Synthesis", is very brief and summarizes the general timidity in approaching any conceptual development. With other recent books, such as R.V. Ruhe's *Geomorphology* (Houghton Mifflin, 1975) and P.W. Birkeland's *Pedology, Weathering and Geomorphological Research* (Oxford University Press, 1974), it will nonetheless be of help to those wishing to begin work on a topic which is, and should be, ripe for considerable development. □

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Back to the land

D.L. Dineley

The Stratigraphy of the British Isles, 2nd Edn. By Dorothy H. Rayner. Pp.460. Hbk ISBN 0-521-23452-2; pbk ISBN 0-521-29961-6. (Cambridge University Press: 1981.) Hbk £32.50, \$59.50; pbk £12.50. *The Structure of the British Isles*, 2nd Edn. By J.G.C. Anderson and T.R. Owen. Pp.251. Hbk ISBN 0-08-023998-6; pbk ISBN 0-08-023997-8. (Pergamon: 1980.) Hbk £13.75, \$32; pbk £5.95, \$14.50. *Methods in Field Geology*. By Frank Moseley. Pp.211. Hbk ISBN 0-7167-1293-8; pbk ISBN 0-7167-1294-6. (W.H. Freeman: 1981.) Hbk \$29.95, £12.95; pbk £6.95, \$14.95

GOOD "standard" geological texts abound; trying to revise them to keep abreast of new facts and ideas is a daunting task for any author. In this trio, the stratigraphy and structure of the British Isles are described in two second editions, while the third text shows how basic field studies may be carried out. Whilst the influence of new hypotheses upon the Rayner and the Anderson and Owen books is rather small, Moseley's book is another matter altogether; new ideas have their place there, and in style and content it is very welcome.

Since the first edition of Rayner appeared in 1967, the impact of plate tectonic theory upon stratigraphy has prompted intriguing new scenarios explaining the geological evolution of the British Isles and western Europe. Dynamic stratigraphy has replaced the classical "static" listing of strata; attention has swung to the relationships between sedimentation, igneous activity and tectonism. The first edition was a clear account of the strata found in these islands. The second is



equally elegant and there is reference to plate tectonic influence upon palaeogeography and the stratigraphic record. Such references are, however, so restrained as to seem reluctant. Perhaps because we now have so many working hypotheses in this area, Dr Rayner has deliberately avoided them and has confined herself to making few changes to her previous account. The individual stratigraphic trees have not lost their identity in the collective crustal evolutionary wood. The diagrams are clear and good — many more of the same standard would have been welcome.

The teaching of stratigraphy now involves emphasis upon correlation and interrelationships of many different kinds of phenomena; essential in all of this is the use of fossils. Without palaeontology stratigraphy will fail, and the reference to biostratigraphy in this text remains far too meagre. Lists of strata commonly are as short of meaning as are lists of fossils to most students, but it ought not to be so — here is a missed opportunity to remedy the one substantial criticism that I had of the original edition. All in all, however, the book is adequate for the undergraduate “who may have only a general acquaintance with the broad principles of geology”.

Structural geology has made some significant strides since the first edition of Anderson and Owen's book was published in 1968, and not only in the field of plate tectonics. Ideas about the formation of sedimentary basins and about both shallow and deep geological structures in Britain are changing and debate is lively. This text has not gone as far as might have been expected in presenting new ideas and integrating them with previous knowledge. It remains an orthodox description of structural units, one by one, grouped as Precambrian, Caledonian, Hercynian and Alpine terrains, Tertiary igneous terrain and with a chapter on “The Seas around Britain” (an unfortunate and inaccurate title) that contains material that should have been integrated into the preceding chapters. The geographical distinction between structures on land and those on the submerged continental shelf is, to an increasing extent, untenable.

Chapters 2 and 3 deal with the tectonic evolution of the British Isles. A simpler outline of palaeogeographical changes might have served better at this stage, with a fuller, more decidedly plate-tectonic-orientated discussion at the end of the book. The style of this volume is very dry; the illustrations are generally poor and many are very poor indeed. The authors advise that the book should be read with the aid of the appropriate geological maps published by the Institute of Geological Sciences, but there are very few pointers in the text as to which maps should be consulted regarding specific features or terrain. The list of references is extensive, but the standard bibliographical abbreviations are not used and several

entries are inaccurate. The printing, direct from authors' typescript, the errata and the poor diagrams give the book the air of having been compiled in a great hurry. One would have expected better.

A book to turn students' attention and interest towards how (a local) stratigraphy or structure is discovered, examined and recorded seems doubly welcome in the context of what has been said above. Hail, then, Moseley's students' manual of field geology! Principles of geological mapping are nicely stated and readable case histories are provided to illustrate them. The latter cover diverse terrains, from Greenland's icy mountains to the seared wastes of Oman, with Cumbria, Andalusia and even a Quaternary torment in between. Igneous,

metamorphic, sedimentary and surficial geological examples are treated with just about the right level of technicality and with commendable care. The book shows how aerial photographs, field survey and data logging of many kinds are pressed into map-making. Abundant and generally excellent illustrations complement the text very well.

This is an attractive book and something of a bargain, especially since it seems to capture the essence and spirit of geological exploration. Congratulations to author and publisher for a brave new book. □

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Geological classics

K.G. Cox

Basalts and Phase Diagrams: An Introduction to the Quantitative Use of Phase Diagrams in Igneous Petrology. By S.A. Morse. Pp.493. ISBN 0-387-90477-8. (Springer-Verlag: 1981.) DM62, \$36.60.

IN THE teaching of igneous petrology the study of phase diagrams for compositionally-simple synthetic systems occupies an honourable niche similar to that of Latin in the arts subjects. It is thought to be good for the soul, though the precise benefits are sometimes difficult to define.

Professor Morse's book is probably the most comprehensive survey available for all those systems which are relevant to the study of natural igneous rocks, particularly though not exclusively, basalts. It is written with clarity, humour and a loving attention to detail. The diligent reader, undergraduate or postgraduate, will acquire a good feel for the way igneous processes work, by absorbing the many, but inevitably rather diffuse, parallels between synthetic and natural systems. Our old friends such as Ab-An-Di, Fo-Di-SiO₂, the ternary feldspars and many more, are dealt with in detail, and there are good sections on the effects of volatiles and the interpretation of layered intrusions. On the level of simple, every-day problems of how magmas crystallize, fractionate or originate by partial melting, this is an excellent book.

I am less happy when the author becomes involved with more major issues of basalt genesis. The decision to restrict discussion to synthetic systems means that problems arising from work on natural materials (basalts and peridotites), and no less from geochemistry and field studies, cannot be adequately dealt with. In itself this might be acceptable — any author has to decide what to leave out — were it not for the misleading nature of some of the

comments which do appear. I doubt, for example, if many petrologists really would agree that “the heart of the basalt problem lies in the eruption, often from the same vent, of tholeiitic basalts at one time and alkali basalts at another time” (p.2), though they might have in the mid-1960s. Other examples, which are consistent with this rather dated viewpoint are numerous, and constitute something of a hazard to the incautious reader. Nevertheless, as a book on phase diagrams this is first rate — it is only the basalts which should be taken with a pinch of salt. □

K.G. Cox is a Lecturer in Petrology at the University of Oxford.

Plants of the past . . .

Keith Allen

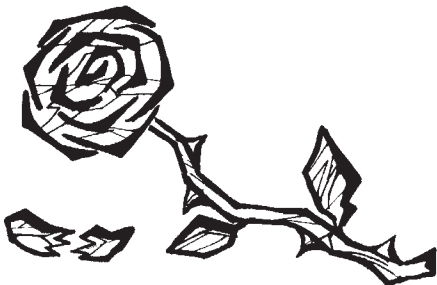
Paleobotany: An Introduction to Fossil Plant Biology. By Thomas N. Taylor. Pp.589. ISBN 0-07-062954-4. (McGraw-Hill: 1981.) \$41.25, £22.95.

I HAVE awaited this publication with much interest, because it is the first general palaeobotany textbook to be written in the English language for almost 20 years. During that time, new discoveries, new interpretations and a much wider use of both stereoscan and transmission electron microscopes have greatly increased knowledge of fossil plant morphology and anatomy. Interest in the origins of plant life, the earliest land plants, the evolution of flowering plants, fossil fuels and the palynological correlation of strata has sparked an awareness of palaeobotany which now reaches far beyond university botany departments.

In writing a palaeobotany textbook, one must choose either to make it very botanical, giving comprehensive

taxonomic coverage throughout the plant kingdom, or to aim for a wider audience, especially amongst geologists, by reducing the morphological information and including details of floras, palaeogeography, palaeoclimatology, fossil fuels and palynology. Like most earlier authors, Taylor has chosen the former course.

This is a scholarly, well-researched textbook, bringing the reader right up to date on plant groups, from bacteria and fungi through to angiosperms. There are also



chapters on methods of preservation and techniques for studying fossil plants, on Precambrian biology and a particularly good one on origins and evolution of the seed habit. Taylor's own research interests are reflected in a fuller treatment of the Pteridosperms, though all groups are adequately covered. In the angiosperm chapter, he has wisely forsaken the taxonomic approach and dealt more broadly with selected topics, including the possibility of pre-Cretaceous angiosperms. The bibliography, usefully listed by chapter, contains over 1,000 references.

Taylor's research papers are always accompanied by excellent illustrations, and he must be aggrieved at the poor production of many of the photographs in the book, some of which are so ghostly that they fail to show clearly the details intended. Also, the positioning of many illustrations leaves unnecessary blank areas which could usefully have been filled with photographs at larger magnification. The photographs of "extinct" palaeobotanists add a nice touch, though I would have liked to see Professor Lang in the Hall of Fame, having contributed so much to Devonian floras and in particular to that from the Rhynie Chert.

To whom do I recommend this book? Clearly all palaeobotanists will find it valuable in bringing them up to date on groups outside their main fields of interest; for this, I find it excellent. Third-year botany students, though perhaps being slightly overwhelmed by the number of genera included, will find it a most useful and readable source of information. I cannot, however, recommend it with too much enthusiasm to geologists, who will find the comprehensive morphological approach very hard going. However, this informative textbook replaces many of the earlier palaeobotanical books, and should be available in all biological libraries. □

Keith Allen is Senior Lecturer in Botany at the University of Bristol.

... and the present

Peter D. Moore

100 Families of Flowering Plants. By Michael Hickey and Clive King. Pp.567. Hbk ISBN 0-521-23283-X; pbk ISBN 0-521-29891-1. (Cambridge University Press: 1981.) Hbk £27.50, \$66; pbk £9.95, \$19.95. *The Study of Plant Structure: Principles and Selected Methods.* By T.P. O'Brien and M.E. McCully. Hbk ISBN 0-632-00926-8; pbk ISBN 0-632-00927-6. (Blackwell Scientific: 1981.) Hbk £20; pbk £12.

ATTEMPTS are being made to shed the Victorian image of botany in many universities, sometimes even resulting in radical, but rarely fruitful, alterations in departmental titles in order to attract more students (Plant Science or Plant Biology, for example). Courses offered to plant-orientated students are also changing rapidly, often in the direction of sub-cellular studies or, at the whole-organism level, towards physiology and ecology. Subjects which once occupied a substantial part of the undergraduate curriculum are now becoming neglected, amongst which are classical (structural) taxonomy and anatomy.

An unfortunate consequence of this is that we are spawning a generation of ecologists which is incapable of defining the criteria upon which plants are identified in the field, and of physiologists which is often sadly deficient in a knowledge of the anatomical structure of the plants with which they deal. In the long term this problem can only be dealt with by a reassertion of balance in undergraduate training, and the two books under review here are designed to encourage the development of such courses.

Hickey and King's book, as its title implies, covers 100 plant families, devoting about four pages to each (up to eight pages for more complex families such as Compositae and Gramineae). For each family one is given general information such as distribution (but no maps), general characteristics (most of which are available from a good flora), economic or decorative uses, and taxonomic subdivision. Thus far the book is comparable to V.H. Heywood's *Flowering Plants of the World* (Oxford University Press, 1978), but is more detailed in its coverage of family characteristics and rather less so in dealing with uses.

A typical member species of the family (or sometimes more than one for families with a wide spread of structural variation, as in the Rosaceae), chosen for ease of availability for student classes (alternatives are suggested), is then fully described and the descriptions are accompanied by detailed line drawings. These will provide valuable aids to students as they dissect the flower and fruit. Extensive annotation is provided on the pages opposite these

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drawings, but a more helpful approach would have been achieved by adding labels to the diagrams. In the seeds of the Violaceae, for example, one can talk of elaiosomes and their function in dispersal, but it would help to have a detailed drawing with an appropriate label. The diagrams are, however, generally well chosen and informative; they lack the striking, aesthetic appeal and full vegetative detail of those in Heywood's book, but they make up for this in precision and clarity.

This book will become a vital and valued tool to those botanists, both amateur and



professional, who wish to look more closely at the structure of easily available flowers.

O'Brien and McCully's book is also concerned with observational techniques, but is evidently intended both for teaching and research. It is essentially a laboratory manual of microscopic, anatomical and morphological methods, giving both theoretical background and practical instruction. It begins from first principles of microscopy and has a "cook-book" approach with some very helpful and informal marginal comments, such as, "If you cannot budge something with finger and thumb . . . before exerting your strength, ASK!". Very clearly aimed at the undergraduate! This chatty style, accompanied by lucid explanations of principle and practice should prove very helpful, especially in such areas as phase contrast and interference contrast microscopy. The language used avoids unnecessary jargon and has many hints for trouble-shooting and image improvement. Chapters on specimen preparation and microphotography for both light and electron microscopy are included. However, the format and sub-heading numbering demand serious criticism; what is otherwise an agreeably usable book is given an unduly forbidding appearance by a complex cross-referencing system and an ugly off-set litho production.

Both of these books seek to reach a small, and still shrinking market. They will serve this market well, but whether they will succeed in reversing its slow demise is questionable. □

Peter D. Moore is Senior Lecturer in the Department of Plant Sciences, King's College, University of London.

Panglossian botany

J.L. Harper

Physiological Plant Ecology, 2nd Edn. By W. Larcher. Pp.303. ISBN 0-387-09795-3. (Springer-Verlag: 1980.) DM59, \$34.90. *Environmental Physiology of Plants*. By A.H. Fitter and R.K.M. Hay. Pp.355. Hbk ISBN 0-12-257760-4; pbk ISBN 0-12-257762-0. (Academic: 1981.) Hbk £19.60, \$47.50; pbk £7.95, \$19.50.

"PLANT ecophysiology, a discipline within plant ecology, is concerned fundamentally with the physiology of plants as it is modified by fluctuating external influences" (Larcher). "How do the intricate physiological mechanisms which have been elucidated over the last 100 years by plant physiologists really operate under natural conditions?" (Fitter and Hay). These sentences define the scope of these two books and at the same time relegate the rest of plant physiology (that which is not "eco-") to the study of plants in constant and perhaps irrelevant conditions.

It is easy to recognize a text in environmental or eco-physiology by the high frequency of the words "adaptation" and "stress". Counting their occurrences and guessing their meanings in different contexts is a fascinating exercise. I suspect that to most ecophysiolgists adaptation is that feature of an organism that may be explained away as "a good thing". It is, then, a comfort that the word carries the guesstimate that such properties are the result of all-powerful natural selection which has sought out the optimal solutions to the problems set by nature. It then remains the task of the ecophysiolgist to demonstrate just how everything about his organisms is for the best in the best of all possible worlds.

Stress is, of course, a "bad thing". Sometimes it is a nasty stimulus, sometimes an uncomfortable response. Fitter and Hay don't like the word because it lacks the precision given to it in mechanics — yet, use it 22 times on one page. (Stress is not in the index, though strain is.) To Fitter stress appears to be a stimulus, a force imposed by the environment, but to his co-author the word appears to mean response — a state induced in the organism: "Plant cells . . . at a turgor pressure lower than the maximum value are said to be suffering from Water Stress". To Larcher "stress is the exposure to extraordinarily unfavourable conditions" — clearly to him it is a stimulus, not a response and must be extreme. I also puzzled about Fitter and Hay's phrase "low light stress" but suppose it is a synonym for "shade stress"!

These two books cover very similar ground — the responses of plants to light, water, nutrients, temperature (and for Fitter and Hay the effect of animals and some microorganisms). Fitter and Hay are more interested in cellular and biochemical processes than Larcher, who is more con-

cerned with the whole plant and its contribution to community processes. Fitter and Hay include a section on allelopathy in which they accept uncritically some very dubious evidence.

Both books carry an extensive bibliography and between them cite more than 1,500 references, of which only about 50 are quoted in both. Together they give a splendid introduction to the literature. Fitter and Hay's is a very Anglo-American text (less than 5% of the references are in alien tongues). Larcher's bibliography is international, as befits an author who advises "Despite the difficulties it is worth taking the trouble to read original works in that language in which the author can express himself most precisely". Is this a warning to those who are reading his own book in its excellent translation?

Students could be expected to read and enjoy chapters in Larcher and not become too badly infected with teleology and Panglossian optimism. However, as regards Fitter and Hay, I admit to a bias: I cannot stomach phrases such as ". . . respiration has a purpose . . ."; ". . . the various mechanisms that have been adopted to fit the species [sic] to their environment"; "plants appear to use it [phytochrome] to detect shading"; "species [sic] . . . may need to maximise plastic responses to permit offspring to colonise a wide range of habitats . . .". I must stint my praise for a book that seems to present biology as a study of purposeful and soothsaying species like football managers planning their future goals. □

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Renewed growth of plants

James F. Sutcliffe

Growth and Differentiation in Plants, 3rd Edn. By P.F. Wareing and I.D.J. Phillips. Pp.343. Hbk ISBN 0-08-026351-8; pbk ISBN 0-08-026350-X. (Pergamon: 1981.) Hbk £20, \$40; pbk £9, \$20.

THIS popular textbook, which was published first in 1970 and reprinted several times before the second edition appeared in 1978, has now had another face-lift. Apart from a shortening of the title — which does not indicate any change in approach — there is a new format with double-column printing on a larger folio size. Even the flowering shoot of *Plumbago indica* which continues to decorate the front cover appears to have had a new lease of life. The plant has turned green again and several new leaves have appeared.

The increase in the size of page has made it possible to achieve a more pleasing layout, and despite the use of a larger typeface and slight expansion of the text, there is a reduction in the number of pages. Unfortunately the improved appearance of the book is not enhanced by the photographic reproductions, the quality of some of which (for example Figs 1.10, 3.4 and 6.1) seems to have deteriorated progressively in succeeding editions.

The sequence of topics follows closely that of the second edition but the chapters have now been arranged in four sections: Structural and morphological aspects of development (Chapters 1 and 2); internal controls in plant development (Chapters 3–6); environmental control of development (Chapters 7–12); and, molecular and general aspects of differentiation (Chapter 13). Each section is prefaced by a short introduction and these serve to link the book together. The reading lists at the end of each chapter have been expanded considerably by the inclusion of some recent reviews and research publications.

However, much of the text remains un-

altered and the claim of the publisher that the book has been "extensively re-written" is hardly justified. New material has been added here and there, particularly in the chapter dealing with the biochemistry of growth-regulating substances. Despite continuing research we are still a long way from understanding the mechanism of their action. In fact, as the authors recognize, the picture instead of becoming clarified seems to be getting more confused — a cause of some embarrassment to those of us who attempt to teach this subject to biologists.

Librarians who stocked up recently with copies of the second edition of this book will be relieved to learn that there is no great need to replace them with the new edition. Undergraduates who have little choice may begrudge the extra cost, but we will go on recommending the book to our students as the best overall introduction to the physiology of plant growth that is available at present. □

James F. Sutcliffe is Professor of Plant Physiology at the University of Sussex.

Introducing whom to natural products?

T.W. Goodwin

Secondary Plant Metabolism. By Brian Vickery and Margaret L. Vickery. Pp.335. Hbk ISBN 0-333-27017-7; pbk ISBN 0-333-27018-5. (Macmillan Press, London/University Park Press: 1981.) Hbk £25, \$49.50; £12.

It is important not to criticize a book for not being what it was never intended to be. This is made difficult in the present instance because neither the authors nor the publishers give any hint of the type of reader the book is aimed at. What the book clearly is, is a full and up-to-date descriptive account at a rather simple level of the formation and metabolism of secondary plant products. On a slightly lower level, the function, uses, ecological interactions and chemosystematic properties are also discussed.

After an introductory chapter, which considers such topics as the differences between secondary and primary metabolites and how biosynthetic pathways are elucidated, the bulk of the book is devoted to essays on families of compounds which arise from basic precursors: these are sugars and sugar derivatives arising from the primary product of photosynthesis, considered to be glucose 6-phosphate; the various fatty acids produced from the acetate-malonate pathway and their incorporation into plant lipids and glycerides; polyketides from acetate which are mainly fungal rather than higher plant products; terpenoids derived from

mevalonate; shikimic acid derivatives; compounds synthesized from two different fundamental units as exemplified by the flavonoids; porphyrins, purines and pyrimidines; compounds, including alkaloids, which are derived from various amino acids. A great deal of ground is covered and one's way about the concentrated information in the book is facilitated by three good indexes.

One can make a reasonable guess that this book is aimed at organic chemistry students studying a natural products course in their second year. It would be a satisfactory basis for such a course but probably lecturers would nowadays concentrate more on reaction types and reaction mechanisms rather than simply on biogenetic schemes. Certainly, stereochemical aspects would be given more prominence than they are afforded in the present text. The book would be useful to biochemists for basic information but not as a working text because, as the authors state, enzymology — the ultimate key to the explanation of all biosynthetic activity — is not included. In the same way it would be useful as a source book for botanists, agriculturalists and pharmacists.

This, then, is a good introductory book on natural products, although there is difficulty in visualizing exactly which academic slot it is intended to fill. □

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Botany for beginners

Ralph S. Quatrano

Biology of Plants, 3rd Edn. By Peter H. Raven, Ray F. Evert and Helena Curtis. Pp.686. ISBN 0-87901-132-7. (Worth: 1981.) \$19.95, £10.50.

ONE OF the most widely adopted textbooks for introducing students to the plant sciences over the past several years has been *Biology of Plants* by Raven, Evert and Curtis. The new and much improved third edition of this popular text must still be considered as one of the best overall books of general botany.

Previous editions were clearly aimed at beginning courses for majors. Such courses would form the basis upon which more advanced courses, such as morphology, physiology and ecology, would build. The new edition is no exception. The depth and breadth are still sufficient for majors and it will be a good source book for any student of the biological sciences. However, the detail is not overwhelming and with the continual mention of applied and practical aspects of plant biology, as well as good chapter summaries, appendices for chemistry and classification, and a complete glossary, makes it a possible choice for non-major courses. Much of the condensing and rewriting in this edition have also helped to make it an attractive choice for a less rigorous introduction to botany. The length of the text, however, may be excessive for such courses, especially in view of the availability of other shorter, good "non-major" texts (see *Nature* 284, 91; 1980).

Although the sequence of topics presented in the text may not be followed, most botany instructors will find no problem in choosing specific chapters to fit their schedule. Several changes for the better in the organization of the third edition include moving the chapter on the chemistry of heredity to the section on genetics and evolution, more emphasis on the Cyanobacteria, and the treatment of the fungi as a group separate from the water and slime moulds (Protista). Several sections have been condensed (for example "Ecology", "Genetics and Evolution") without deleting important concepts, while others have been extensively updated and revised (for example "Growth Regulators", "Uptake and Transport").

The illustrations and diagrams are clear and the inclusion of colour plates for angiosperm anatomy in this edition will be helpful to students in the laboratory. The book will undoubtedly maintain its high ranking and competitiveness with other new editions of excellent, popular texts such as the sixth edition of *Botany* by Weier *et al.* (Wiley, 1982). □

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Legacy of tradition in plant geography

Honor C. Prentice

Historical Plant Geography: An Introduction. By Philip Stott. Pp.151. Hbk ISBN 0-04-580010-3; pbk ISBN 0-04-580011-1. (George Allen & Unwin: 1981.) Hbk £12, \$30; pbk £5.95, \$14.95.

This book is an elementary introduction to the traditional subject matter of "plant geography" — species distributions and their interpretation. It is concise, very readable, well produced and accessibly priced. Care has been taken to make it comprehensible to students with different backgrounds in biology, geography or environmental science. There are chapters on taxonomy, maps, distribution patterns, disjunctions, endemism, and geological and historical evidence.

I am sceptical, however, about the value of a textbook on plant geography that carefully avoids modern ecological and evolutionary concepts. Some plaintive remarks on the state of biogeography (Chapter 1) are revealing — if the "ecological tradition" has "flourished and grown out of all proportion" it is surely because of advances in ecological theory!

Classical phytogeography has not made comparable progress, and indeed the same paradoxes and ambiguities that run through the classical literature reappear,

unresolved, in Stott's book. ("Isoflors . . . are used to locate centres of origin and migratory patterns . . ." — but how?) Pollen analysis is introduced, but its most relevant results — reconstructions of complex Late-Quaternary migrational histories — are not. There is no discussion of how taxonomic information may lead to

reconstructions of phyletic patterns; cluster analysis and the like are dealt with, but cladistics is not mentioned, despite the impact of cladistic and vicariance concepts on modern evolutionary biogeography. For many reasons, then, I find this a disappointing book however well it succeeds in its stated aims. □

Honor C. Prentice is a Visiting Research Fellow in the Department of Biology at the University of Southampton.

Ethology: the classic and the avant-garde

Peter Marler

An Introduction to Behavioural Ecology. By J.R. Krebs and N.B. Davies. Pp.292. Hbk ISBN 0-632-00666-8; pbk ISBN 0-632-00668-4. (Blackwell Scientific/Sinauer: 1981.) Hbk £18, \$39.50; pbk £7.50, \$16.50. *Modern Ethology: The Science of Animal Behaviour.* By S.A. Barnett. Pp.705. ISBN 0-19-502780-9. (Oxford University Press: 1981.) £12.95, \$23.95.

ETHOLOGY helped the study of animal behaviour off to a good start with a number of simplifying assumptions, one of which was crucial in convincing sceptics that natural behaviour is even studiable. Given the right kind of description, it was asserted, behaviour would turn out to be as stereotyped and species-specific as morphology. This worked well for a time, but in the 1960s it became obvious that, just as morphology exhibits adaptive variations, so patterns of behaviour vary widely within a species. Even small differences in the behaviour of groups of individuals may be so amplified by processes of social interaction that different patterns of social organization are created. The theorizing of Lorenz and Tinbergen, primarily reductionistic in its orientation, was ill-suited to guide investigations of the quantitative impact of behavioural variations on animal-environment interactions. Counsel was sought from other disciplines such as ecology, population genetics and economics.

In the past ten years several subdivisions of ethology have developed to deal with such problems one of which, behavioural ecology, is admirably reviewed in the introductory text by Krebs and Davies. The approach is that of the evolutionary biologist, concerned with functional explanations of behaviour at the level of individual and kin selection. The book is a pleasure to read. Its style is lucid and entertaining, and the illustrations are clear and instructive. There are well-balanced reviews of the evolution of alternative social systems and evolutionarily stable strategies, feeding strategies and optimality modelling, fighting and

manipulation, altruism and cooperation. The pride of place assigned by Kropotkin and Allee to cooperation, however, is now superseded by an emphasis on conflicts of interest, effectively translating the motivational drive conflicts of Tinbergen into the functional domain. Instead of collaboration, we have selfishness, spite and calculated exploitation.

While arguing their case forcefully, Krebs and Davies are cautious about over-extending their theorizing, much of which, they admit, serves more as description than as predictive theory. In that sense, the book also serves well to point the more ambitious student towards new problems awaiting solution. In particular, genetic issues raised, such as uncertainties about paternity and how "kin" are actually distributed in natural vertebrate populations, deserve to be tackled by direct observation and experiment as well as at the abstract, theoretical level.

Behavioural ecology forms only a part of Barnett's much larger endeavour. *Modern Ethology* is more of a "mechanisms" book than that by Krebs and Davies, analysing critically the concepts of classical ethology in the light of recent research, and reviewing an extensive literature in the process. It is both a textbook and a serious, scholarly work that grapples with conceptually difficult and controversial issues such as intention, conscious awareness and the role of reductionism in behavioural science. Accounts of social behaviour are interwoven with such topics as behavioural endocrinology and learning theory. One of the major index entries, habituation, does not appear in the index of Krebs and Davies, though clearly relevant to their subject matter. In this sense, the two books complement one another well.

Especially salutary are Barnett's discussions of concepts of heritability and aggression. Aggression is interpreted with care, especially in the section on human ethology. A quotation from W.D. Hamilton, a founder of sociobiology and behavioural ecology, stating that "vicious and war-like tendencies are natural in

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man'' sounds a cautionary note. Misunderstandings about such issues are bound to arise until we understand the epigenetics of such traits as aggressive behaviour and the points in development at which they are accessible to environmental modification. As Barnett indicates, the interaction of nature and nurture is a central problem in biology and those trained in the ethological tradition are especially well placed to deal with it. An historic opportunity will be missed if current enthusiasm for behavioural ecology diverts all young ethologists from what has to be the most challenging and momentous issue ethology has yet faced. Only when we understand how genes impose adaptive limits on behavioural plasticity will we fully appreciate the pervasiveness of genetic influences on the development of behaviour, whether animal or human. □

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Sixty years on?

Michael B. Usher

Elementary Mathematical Ecology. By John Vandermeer. Pp.294. ISBN 0-471-08131-0. (Wiley: 1981.) £24.05, \$43.25. *Population Systems: A General Introduction.* By Alan A. Berryman. Pp.222. ISBN 0-306-40589-X. (Plenum: 1981.) \$16.95, £10.71. *Population Ecology: A Unified Study of Animals and Plants.* By Michael Begon and Martin Mortimer. Pp.200. Hbk ISBN 0-632-00812-1; pbk ISBN 0-632-00667-6. (Blackwell Scientific/Sinauer: 1981.) Hbk £15, \$33; pbk £7.90, \$16.95.

MANY origins of the subject of population dynamics can be traced to the 1920s, when A.J. Lotka, R. Pearl, P.F. Verhulst, V. Volterra and others were publishing relatively simple mathematical models of population growth and interaction. During the following half-decade the subject grew slowly, but in the 1970s there was an explosion of research interest. It is inevitable that this would eventually manifest itself in teaching and writing textbooks to fill the students' need. Three recent examples are considered here.

John Vandermeer teaches population dynamics with a programmed text of ten lessons, each of which is designed to take 5 ± 2 hours. The explanatory sections of *Elementary Mathematical Ecology* are kept short, and the exercises, for which relatively sketchy but adequate answers are given, are both numerous and essential for understanding the lesson. I am reasonably

certain that most undergraduates would take considerably longer than the designed time, and in the exercises I found the lack of consistency of rounding intermediate results most confusing (for example, in the first chapter one has $\ln(2.25) = 0.81093$ and $\exp(0.83) = 2.3$).

In *Population Systems*, Alan Berryman has written a more traditional textbook, setting the subject out in only six chapters which contain few exercises and only brief solutions. The text itself does not make reference to the ecological literature: a series of notes, listed in rather small type at the end of each chapter, provides reviews of a few references and also gives some of the more technical material. I found the shuffling between text and notes a rather frustrating aspect of the book's subject presentation.

A slight departure from the usual progression from single-species to two-species interactions to communities is a characteristic of Michael Begon and Martin Mortimer's *Population Ecology*. Although the first five chapters follow the first two steps in this sequence, the authors conclude the book with three chapters — on life-history strategies, population regulation and community structure — in a section called "Synthesis". Although this makes for interesting, if unexpected, reading, since one is asked to think about migration, costs of reproduction, r- and K-selection, and so on, after reading about interspecific competition and predator-prey relationships, the attempt at synthesis really fails.

It is interesting to compare the subject matter of these books. All of them include introductions to population growth, interspecific competition, predator-prey relationships, spatial patterns (rather briefly dealt with in *Population Ecology*) and community structure (only sketchily introduced at the end of *Elementary Mathematical Ecology*). A consideration of life tables is the most surprising omission from *Population Systems*, which also lacks an account of matrix models of age-structured populations. These, then, are the core topics, but what are the specialities? *Elementary Mathematical Ecology* contains data on island biogeography and species-area relationships, but I wish that the author had not used the ambiguous term "species number" (implying ranking) for the unambiguous "number of species". *Population Systems* takes a systems analysis approach, introduces much of the jargon such as "feedback", which then leads to an extensive consideration of density-dependent effects. *Population Ecology* includes chapters dealing with life-history phenomena, key factors and harvesting. None of the books contains detailed discussions of genetical aspects of population regulation.

The theoretical foundation of 60 years ago is still strongly evident. *Population Systems* perhaps diverges most in taking a

systems theory point of view. But, for teaching, I would certainly recommend the excellent blend of example and theory in *Population Ecology*, and possibly support some areas by the greater theoretical and practical development coming from the 291 exercises in *Elementary Mathematical Ecology*. □

Michael B. Usher is Senior Lecturer in Biology at the University of York.

Search for a science

Graham Hoyle

Neuroethology: An Introduction to the Neurophysiological Fundamentals of Behavior. By Jorg-Peter Ewert. Pp.342. ISBN 0-387-09790-2. (Springer-Verlag: 1981.) DM 49, \$29. *Neuroethology: An Introduction.* By D.M. Guthrie. Pp.221. Pbk ISBN UK 0-632-00303-0; ISBN US 0-471-26993-6. (Blackwell Scientific/Halsted: 1981.) £8.75, \$27.95.

THESE two introductory texts, appearing independently at about the same time, offer an insight into viewpoint as well as subject matter. Ewert does his research on a backboned animal, the toad; Guthrie on invertebrates. Each goes out of his way to offer a fair balance, with extensive examples from the other's group. Many of the same studies are reported by both authors, showing that they agree as to what constitutes neuroethology. It is remarkable to find such concurrence, since others who consider themselves to be neuroethologists have quite different notions as to what should be included. Guthrie has chapters on evolutionary trends and human neuroethology, with illustrations taken directly from textbooks on neuroanatomy, psychology and animal behaviour. Ewert avoids such a nebulous search for principles, sticking to observations and hypotheses — although he does manage to squeeze in a section termed "Stress in Human Society".

The Nobel prize-worthy finding of Lorenz and Tinbergen in the name of ethology was a simple generalization: complex behavioural acts of animals as different as insects, birds and human beings are handled genetically — and therefore evolutionarily — as wholes. This applies especially to acts of territoriality, home-building, prey-capture and courtship. They introduced key concepts and clear descriptive terms, notably "fixed action pattern", "releaser", "drive" and "consummatory act". Each of these offers a major challenge to neurobiologists, because each implies a specific kind of underlying neural circuitry and properties. Working them out offers a splendid and significant intellectual/laboratory pursuit,

but there have been only a very few successes in this new field so far. Ewert and Guthrie both cover them, but all too briefly. To create a larger body of knowledge, each has padded the relevant data with selected bits of physiology, psychology, sociology and ethology. Thus students will be able to learn from either of these books, but only if they also receive lectures and extensive reading assignments, and if they already have a good background in basic neurophysiology. Both books are lavishly and attractively illustrated; Ewert's, in particular, is well-endowed with clear diagrams, many of which are original, in two colours. Each represents good value for money.

Unfortunately, the uninitiated reader is very likely to suffer from a severe attack of

mental indigestion because of the highly condensed treatments of diverse sets of data. The budding researcher will still have to make a critical choice within some traditional discipline before being able to practise neuroethology, and will be forced to face the difficult decision as to whether to go with backbones, or a "squishy" or "crunchy" invertebrate. In effect, this is a choice between going for cellular detail now, or for the prestige and significance automatically associated with vertebrate animals, but which at present show few signs of yielding the information needed for understanding at the level of nerve cells and circuits. □

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Strong-minded neuroscience

Maria Fitzgerald

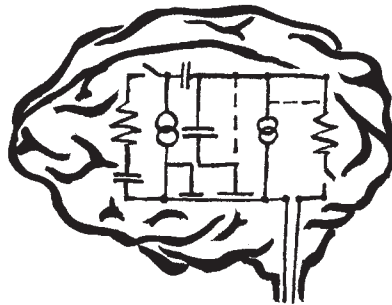
Principles of Neural Science. Edited by Eric R. Kandel and James H. Schwartz. Pp.731. Hbk ISBN 0-7131-4405-X; pbk ISBN 0-7131-4406-8. (Elsevier/North-Holland/Edward Arnold: 1981.) Hbk £45, \$60; pbk £25.50, \$32.50.

NEUROSCIENCE is one of the most rapidly expanding branches of biology today; the task of putting together a comprehensive textbook on the subject is therefore becoming increasingly difficult. Such a book should inform clearly and accurately of the basic scientific facts while giving a balanced account of newer, less substantiated work. Ideally it should also convey the excitement of new ideas and the importance of future research. *Principles of Neural Science* fulfills all of these requirements. It is an excellent book, fun to read yet informative and accurate — a combination rare in scientific literature.

The editors believe that it is implicit in most neuroscientists' thinking that "all behaviour is an expression of neural activity". The task, therefore, of the neuroscientist is to explain how the brain and its cells function and so to understand behaviour. This view is the underlying theme of the whole book and it contributes greatly to its strength. It results in a great emphasis on behaviour, from simple spinal reflexes to the neural basis of emotions. It also results in a strong emphasis on disease and mental illness. After all, if behaviour is a reflection of brain function, then disorders of affective and cognitive functions must result from disturbances of the brain, and much can be learnt from them. Some may disagree with this philosophy and thus with the framework of the book; nevertheless they will still find the contents useful and stimulating.

All of the expected chapters are there, from cellular membranes to motor control,

plus good, up-to-date sections on topics such as sleep, ageing and CNS development. In each section, the relevant regional anatomy is surveyed, which is especially useful in discussions of the brainstem and the limbic system. At the end of the book there are several excellent appendices on brain fluids, neuro-ophthalmology and electrical circuit theory with set questions to work through.



Of course there are shortcomings. Neurochemistry, one of the most challenging branches of neuroscience, is inadequately covered. The basic, classical information is there but neuropeptides, for example, get only cursory attention. The autonomic nervous system is mentioned now and again but should certainly have had its own chapter. The index is so short as to be practically useless, although in compensation the chapters are clearly divided by straightforward subheadings. These are in the American style of a statement of fact; some are refreshing in this cautious age, some irritating. My particular favourite, arbitrarily used in the chapter on diseases of neuromuscular transmission, is: "Unsolved problems remain". □

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Childish thoughts

Albert Yonas

Foundations of Developmental Psychology. By R.C. LaBarba. Pp.545. ISBN 0-12-432350-2. (Academic Press: 1981.) \$20.75, £13.20. *Developmental Psychology*, 3rd Edn. By R.M. Liebert and R. Wicks-Nelson. ISBN 0-13-208256-X. Pp.665. (Prentice-Hall: 1981.) \$21.95, £16.45.

I DO NOT know how many students enroll in introductory courses in child psychology each year, but the number must be vast. Apparently, every publisher of textbooks feels that it is worthwhile to produce a new book, or at least a new edition of an old one, every two or three years in an attempt to attract part of this huge market. Something like 80 to 100 introductory child psychology textbooks are currently available. This competition has resulted in some specialization of books, as well as a great deal of homogeneity in the content and organization within each type of book.

Ten years ago, many leading texts tried to do everything. They were organized by age, beginning with prenatal development and infancy, and describing the child up to adolescence. In addition, within each age section, topics such as perceptual and social development were discussed. The writing style of many of these books has always reminded me of a sequence of 3" x 5" note cards.

In recent years, specialization has led to the creation of a great many texts that do little to introduce students to the scientific discipline of child psychology; rather, their goal is to give the prospective parent or teacher a picture of what the average child is like at each stage of development and to provide information of practical use. At the opposite end of the continuum, we have LaBarba's *Foundations of Developmental Psychology*. This book is a scholarly and historical introduction to the theoretical issues that have guided developmental researchers. It presents empirical studies primarily to illustrate issues and concepts, and makes little attempt to give the reader a picture of what behaviour to expect from a newborn child or to describe the average two-year-old.

The book is organized topically, with a heavy emphasis on biological areas such as genetics and human behavioural embryology. The behaviour of the neonate apparently is not of sufficient interest to the author to merit inclusion. Very little space is given to the development of sensory and perceptual processes; there is no mention, for example, of any sensory modality other than vision.

Unlike most introductory books, LaBarba's presents theoretical and empirical work not as fact but as the focus of argument with the opposing point of view. This may confuse and depress students, although it does convey the state of the art.

The basic problem with this text may be its assumption that a unified discipline of developmental psychology exists with its own explanatory concepts and body of facts that can be presented in an introductory text in the same manner as would a physics text. In reality, developmental psychology is an interdisciplinary field, strongly concerned with practical applications. It focuses on the description of the course of development, and is still groping towards theoretical concepts which will make explanation possible.

Developmental Psychology, by Liebert and Wicks-Nelson, is the third edition of a comprehensive and successful text that should be even more successful in this new version. The authors view developmental psychology as a branch of science that is both basic and applied; they believe that students can best understand practical applications in the light of the theory and research on which they are based.

The authors and publisher have responded to competition by producing a book with every pedagogical aid imaginable, from an outline at the beginning and a summary at the end of each chapter, to coloured and italicized marking of important concepts and terms. The figures are especially effective.

The quality of the writing is probably the book's most outstanding trait. It has fewer details, and is more readable, than a similar, widely used book by Hetherington and Parke. Students are introduced to basic research with as little pain as possible; interest is gained by discussion of practical topics (for instance, various childbirth methods). The sense of controversy and uncertainty in developmental psychology is almost entirely missing from this book, but this may well be pedagogically necessary.

While many teachers may find LaBarba's book full of fascinating information useful in the preparation of lectures, they are more likely to assign Liebert and Wicks-Nelson's book to their students. □

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Merit in a minefield

B. Burnet

Behavior Genetics and Evolution. By Lee Ehrman and Peter A. Parsons. Pp.450. ISBN 0-07-019276-6. (McGraw-Hill: 1981.) \$31.50, £17.50.

AS A focus of intellectual excitement, the genetics of behaviour is rivalled only by pursuit of an understanding of the genetics of differentiation and development. Since it requires an interdisciplinary approach in integrating biology and genetics with social sciences, it is also a minefield for

misunderstanding, and requires a nice balance of scholarship and careful judgement in any who would venture a good textbook for teaching. Here is just such a text. *Behavior Genetics and Evolution* is a revised and updated edition of *The Genetics of Behavior* published a few years ago by the same authors. It gives a comprehensive account of the principles and methods used to study the genetics of behaviour and stresses the point that greater understanding of the genetic architecture of complex behavioural traits necessitates subdividing them into discrete components prior to genetic analysis. Separate chapters are devoted to reviewing the genetics of behaviour of human beings, small mammals and fruit flies, together with a range of other organisms including some less familiar invertebrates and domesticated species.

The authors give a balanced account of the genetics of human behaviour and, rightly in my opinion, emphasize the need for concentration less on the current obsession with IQ differences than on the genetic analysis of more accessible human sensory, perceptual and motor skills — although, in view of the difficulties

inherent in interpreting EEG wave patterns, speculation about their possible significance with regard to deviant or criminal behaviour should be treated with caution. Particularly valuable is the discussion of the complications caused by cultural transmission in relation to the inheritance of human behavioural characteristics. In the later chapters the special merits of the book emerge in a synthesis discussing the genetic architecture of behaviours in relation to niche breadth of species, habitat selection, population structure and mechanisms of evolution. Relatively little attention is given to kin selection and the evolution of altruistic behaviour, which is surprising in view of the widespread current interest in this topic.

The book is directed towards undergraduate and postgraduate students in biology and psychology, and should also be valuable to those professionally involved in the social sciences, agriculture or medicine who require a comprehensive treatment of the subject. □

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Two views of evolutionary genetics

J.S. Jones

Basic Population Genetics. By Bruce Wallace. Pp.688. ISBN 0-231-05042-9. (Columbia University Press: 1981.) \$26, £12.95. *Introduction to Quantitative Genetics*, 2nd Edn. By D.S. Falconer. Pp.340. ISBN 0-582-44195-1. (Longman: 1981.) £9.95, \$23.

THE old controversies about Darwinism have made their periodic return to the public stage. In modern terms, evolution is nothing more than a change in gene frequencies. Here are two books dealing with genes in populations; one, that of Wallace, is concerned largely with natural populations, the other mainly with genetic responses of populations in the laboratory or on the farm.

Wallace's substantial work surveys many aspects of population genetics. After a chapter on the usefulness of models (which are used throughout the book, but always at a level which should be accessible to undergraduates), he considers attempts to measure genetic variation, the effects of migration and assortative mating on population structure, the influence of inbreeding and mutation, and modes of selection in field and laboratory. An account of genetic load (a term which has lost much of its earlier prominence) leads into a treatment of heterozygote advantage, which is considered in the context of "hard" natural selection (which depends on the rigid application of

environmental challenges) versus "soft" selection (in which fitness depends as much on the frequency of competing genotypes as on the rigours of the environment). The book concludes with a discussion of coadaptation and population differentiation. Wallace's treatment is biased towards the familiar laboratory species of *Drosophila* — organisms which are unique in the extent of our knowledge of their genetics and our ignorance of their ecology. There is little mention of the classics of ecological genetics such as industrial melanism, mimicry or heavy metal tolerance in plants.

In later chapters Wallace enters into speculation and polemic which, although always entertaining, means that in some ways the book belies its title; this text is much more than an account of the basics of evolutionary genetics.

Falconer has produced a new edition of a book which has for 20 years been the basis for almost all introductory courses in quantitative genetics. His second edition looks as durable as the first. It gives a clear and complete account of the inheritance of continuous characters, many of which are of considerable practical importance. Falconer does not hesitate to discuss the relevance of research on laboratory populations to cattle milk yield or corn production, so that his book will have a wide audience. Two-thirds of the references cited have appeared since the

first edition, and the inclusion of so much new work inevitably means that the book has lost some of its earlier clarity and directness; quantitative genetics has, in common with all other aspects of genetics, gained nothing in simplicity as our knowledge has increased. Nevertheless, this new text is unrivalled as an introduction to the subject.

As Wallace points out, there are parallels between the theories of population genetics and those of economics. In both fields there exist impressive and complex models of the process of change; a huge literature, of increasing mathematical sophistication, deals with their most obscure workings, and at the furthest reaches of these great machines small bands of workers labour to improve still further our understanding of the details of the evolutionary or economic universe. Economics and evolution share another remarkable feature; in each there exist two quite different sets of models which, although largely complete within themselves, provide mutually incompatible explanations of the same phenomena.

The present dominance of one economic model means that academics must now assess textbooks as much by price as by content. In population genetics there is an equivalent controversy between the view that most evolution results from the differential action of natural selection on genetic polymorphisms, and the more radical thesis that much evolutionary change — including even changes in the fossil record — arises from the accidents of sampling. Wallace, in particular, is an evolutionary Keynesian; throughout *Basic Population Genetics* he emphasizes the directing influence of gradual natural selection in controlling the course of evolution. Although Falconer does devote a higher proportion of his space to the influence of random events, he too does not give this view the prominence which it now has in much of the population genetics literature. Evolution by random change still awaits its student text. □

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Mendel meets DNA: the story continued

D.J. Cove

An Introduction to Genetic Analysis, 2nd Edn. By David T. Suzuki, Anthony J.F. Griffiths and Richard C. Lewontin. Pp.911. Hbk ISBN 0-7167-1263-6; pbk ISBN 0-7167-1307-1. (W.H. Freeman: 1981.) Hbk \$24.95, £18.50; pbk £11.95. *Genetics: A Molecular Approach*. By Laura Livingston Mays. Pp.693. ISBN 0-02-378320-6. (Collier Macmillan/Macmillan, New York: 1981.) £15.95, \$23.95. *Genetics: Its Concepts and Implications*. By Anna C. Pai and Helen Marcus-Roberts. Pp.711. ISBN 0-13-351007-7. (Prentice-Hall: 1981.) \$24.95, £18.70.

LAST year, I reviewed here (*Nature* 289, 702–704) seven textbooks of genetics, published in 1980, which were primarily intended for use by undergraduates taking introductory genetics courses. In 1981, three more general genetics texts have appeared and so we have moved in the space of only two years from a state of relative famine to what might be judged to be a glut.

In my previous review, I was allowed to devote space to my own views of how genetics should be taught at this level. These have not changed so I will not repeat them here at length, but I believe that the teaching of genetics is not made easier by a rigid adoption of a historical approach nor by the compartmentation of classical and molecular genetics. However, as with almost all of the 1980 textbooks, last year's were again slaves of history. All, for

example, introduce Mendelian genetics by considering the pattern of segregation observed when, in a diploid tissue, a character difference is due to a pair of alleles, one dominant to the other. In fact, in all three textbooks, the first segregations described are in the garden pea. Once again, the student is expected to cope with diploidy and dominance at the same time as understanding the principles of segregation. All three texts also adopt a "Mendel before molecules" approach, but Mays manages to deal with meiosis, Mendelism, DNA and even the regulation of gene expression, all in her first chapter. She returns to most of these topics later, where her treatment is, of course, more detailed. There is certainly something to be said for this approach, but in Mays's case its adoption has been accompanied by the sacrifice of rigour.

My review has so far implied that the three textbooks are rather similar and it is therefore time that I should redress the considerable injustice that this impression would cause to Suzuki, Griffiths and Lewontin. Although I was disappointed to find that these authors should have adopted such a conventional approach to the teaching of genetics — and that even they should imply that every gene has only two alleles, one dominant to the other, for about 70 pages of their text — of its kind, this textbook is excellent. It is clearly written, well illustrated and, in particular, as its title implies, puts considerable emphasis on the analytical aspects of genetics. Although it adopts a historical

approach, it is up-to-date but, perhaps surprisingly, it is better in this respect on molecular than evolutionary topics. It contains, for example, a clear account of how DNA base sequence can be determined but does not deal with the punctuated equilibria debate. Lewontin's chapters on quantitative, population and evolutionary genetics, although as usual tacked on behind, are just as good as would be expected from a geneticist of his stature. However, most students will find the book's first chapter, which explores the difference between genotype and phenotype, and which was also contributed by Lewontin, difficult and only understandable when they return to it later having acquired some knowledge of the physiology of gene expression.

The book is not without faults; its treatment of the regulation of gene expression in the context of metabolism is weak in so far as it implies that there is much more uniformity of mechanisms than now seems justified and in particular that the operon is universal among prokaryotes and fundamental to gene regulation.

Mays's book, subtitled "A Molecular Approach", attempts to integrate molecular with classical genetics much more than Suzuki, Griffiths and Lewontin. It adopts a descriptive style which therefore neglects the logical rigour which is central to both molecular and Mendelian genetics. It is up-to-date and deals, perhaps in too much detail, with topics such as DNA sequencing. Population, ecological and evolutionary genetics are covered in two chapters, but although one of these is not at the end of the book, there is no greater integration of these topics with the rest of genetics than in most other texts.

Pai and Marcus-Roberts is unfortunately the runt of this litter. Its approach is conventional, very descriptive and where it attempts to explain classical genetical phenomena in physiological or molecular terms, the explanation is often misleading or even wrong. The book is in fact full of errors, some trivial, others more important. *Hemophilus influenza* (sic) is said to be a virus; a heterokaryon "a cell with a diploid nucleus"; an *E.coli* strain developed for safe genetic engineering to have no sites in its DNA "which can be acted on by restriction endonucleases".

All three books contain set problems. Mays contains no answers while Suzuki, Griffiths and Lewontin in their book give answers only to selected problems, although answers to all problems are available in a separate booklet. The answers are as usual rather brief; no doubt publishers will say that space is at a premium but I still believe that very full answers, perhaps to fewer questions, are essential.

Finally, I must compare this year's books with last year's. Suzuki, Griffiths and Lewontin goes straight into my "best buy" category. It is inevitably a little more

up-to-date than my last year's choice, Ayala and Kiger's *Modern Genetics* (Benjamin/Cummings, 1980) but I do not think that it is obviously better. I prefer Ayala and Kiger's somewhat less historical approach and their greater integration of molecular and Mendelian genetics, although I believe both should be taken much further. I still think there is much to be said in favour of Fristrom and Spieth's *Principles of Genetics* (Chiron/Blackwell

Scientific, 1980), last year's second choice, which adopts a much less conventional approach to the subject, and so my own list of recommended texts now includes all these three. Mays's book is neither good enough nor bad enough to warrant special mention but I am afraid I will have to warn my students off Pai and Marcus-Roberts.

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Not quite Wilson

Michael Ashburner

Cytogenetics: The Chromosome in Division, Inheritance and Evolution, 2nd Edn. By Carl P. Swanson, Timothy Merz and William J. Young. Pp.557. ISBN 0-13-196618-9. (Prentice-Hall: 1981.) \$25.95, £19.45. *Cytogenetics: Plants, Animals, Humans*. By Jurgen Schulz-Schaeffer. Pp.446. ISBN 3-540-90467-0. (Springer-Verlag: 1981.) DM69, \$40.60.

A DISTINGUISHED cytologist of my acquaintance never ventures abroad without his copy of the third edition of E. B. Wilson's *The Cell in Heredity and Development* (Macmillan, 1925) under his arm, carefully preserved from the elements by a wrapping of plain brown paper. I fear that neither of the two textbooks of cytogenetics reviewed here will command such devotion 60 years hence. Both Swanson, Merz and Young (SMY) and Schulz-Schaeffer have written texts aimed, by and large, at an advanced undergraduate audience, though both hope that their volumes may be of use to more "advanced" researchers.

The books are not only similar in purpose, they are similar in the general extent of their coverage, treating such broad topics as the structure and function of chromosomes, the behaviour of chromosomes at mitosis and meiosis, chromosome aberrations and chromosomes in evolution in a reasonably conventional way. There is, it is true, a considerable difference in emphasis between SMY and Schulz-Schaeffer; the latter gives the cytogenetics of plants (especially cereals) particularly strong attention, whilst the former devotes more space to a consideration of evolutionary issues. It is, perhaps, for this reason that I am more naturally drawn to SMY and approached Schulz-Schaeffer more from the desire to know what new I could glean from it.

I began this review with an implicit comparison of these two books with E.B. Wilson's masterpiece. Part of the reason for Wilson's greatness (I speak of both book and man) was the regard and understanding he had of genetics. The signal failure of Schulz-Schaeffer stems, in

my opinion, from the absence of any true integration of the "cyto-" and the "genetics". This book suffers too from a lack of balance in the presentation of the material and from the author's inability to distinguish between the trivial and the lasting. Perhaps I should illustrate these harsh criticisms. A very common class of chromosome aberration, both in natural and experimental populations, is the inversion. Inversions have the property that, when heterozygous, they suppress genetic recombination. The precise mechanisms by which this suppression of recombination is achieved were elucidated by Sturtevant and Beadle in 1936, and their paper (*Genetics* 21, 554-604) is one of the most elegant in genetics. Not only does Schulz-Schaeffer fail to give a summary of this work (let alone reference it) but he wastes his reader's time with such trivia as the "fact" that "twenty seven different autosomal inversions have been reported from three different localities in Bulgaria in the mosquito species *Anopheles messeae*".

A second example of the lack of balance in this book may not be amiss. A paragraph (p.294) discussing somatic crossing over includes the statement that "soybean seems to be an ideal object for the study of somatic crossing over". This may well be so, but it is rather an eccentric statement to make when the detailed and sophisticated studies of mitotic crossing over (as it should be called) in both yeast and *Drosophila* have virtually been ignored.

The lack of balance that I see in this textbook is serious: not only will it mislead the student but it will also undermine the confidence of other readers who might otherwise seek from it new information. There is, not surprisingly, much in Schulz-Schaeffer that is new to me, yet I am reluctant to take anything at face value from an author who awards Francis Crick a knighthood (trivial in itself, perhaps, but a minor irritation) and who has, at least on occasion, clearly not consulted his original references. His account of parthenogenesis in aphids is but a poor paraphrase of White's review in the third edition of *Animal Cytology and Evolution*, and I see no signs of any attempt to look at the original data or take into account subsequent studies.

The second textbook, that by SMY, is far better. Although the second edition of a

text first published in 1967, it is really the successor of Swanson's *Cytology and Cytogenetics* published by Macmillan ten years before that. Not only have SMY made a reasonably successful job of integrating the cytology and genetics (though even here I think Swanson did better in 1957) but they have also stamped upon the whole volume an evolutionary perspective that generates an impression of intellectual coherence. There is, however, one area of weakness in this book: that is in the various discussions of molecular cytogenetics. Not only is this subject treated, to my mind, in a rather oversimplified manner but the authors are too naive, accepting at face value some of the more speculative of models whose very authors, I am sure, would shudder to see exhumed in student text. □

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Citron pressé?

P.A. Lawrence

The Genetic Basis of Development. By Alistair D. Stewart and David M. Hunt. Pp. 221. Hbk ISBN 0-216-91161-3; pbk ISBN 0-216-91160-5. (Blackie/Wiley: 1982.) Hbk £18.95, \$27.95; pbk £8.95.

THE genetic approach to developmental biology can be fun and ought to be popular. But embryology is traditionally a soft subject that has often been closer to theology than science, while genetics has always been hard and numerological. Which may explain why, in the past, practitioners of the two disciplines have understood each other little and have rarely collaborated to solve biological problems. This makes no sense at all as genes are there to provide materials and information to construct and maintain animals. Genes and their products ought therefore to be at the heart of embryology — which is just where Stewart and Hunt perceive them to be.

Their aim was to write a brief embryological text from the geneticist's view point, and the result is a usefully analytical account. It is also up to date and breaks with the common practice (especially in embryology) of beating a new textbook out of older ones. In short, the authors have used the primary literature as their source. This may explain why the book is often tricky — especially for the "advanced undergraduate" for whom it is written. And also why it is too much of a review, being strong on lists of experiments and results and weak on explanation. A good example is the description of the bithorax complex of *Drosophila*. This locus is an illuminating link between genetics and development, as it appears that the elements of the locus are specifically deployed in developing segments.

Lewis (*Nature* 276, 565-570; 1978) has shown that without this gene complex most of the segments develop identically and the result is more like a worm than an insect. Unfortunately the account of bithorax by Stewart and Hunt is so replete with facts that this advanced undergraduate found it almost incomprehensible. Nevertheless, the original approach of the book makes it refreshing and, as with a cold drink, one can always leave the ice in the glass. □

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Genetics: bottom . . .

Neville Symonds

Bacterial and Bacteriophage Genetics: An Introduction. By Edward A. Birge. Pp.359. ISBN 0-387-90504-9. (Springer-Verlag: 1981.) DM54, \$25.70.

EDWARD Birge's brief was to produce a book of reasonable size which would serve as a contemporary text for introductory courses in bacterial and phage genetics. This has involved starting with a brief treatment of DNA replication and the nature of mutation, proceeding through chapters on phage genetics and the mechanisms underlying transduction, transformation and conjugation, and then bringing the story up to date with further chapters on plasmids, gene regulation, the molecular basis of recombination and gene manipulation.

The "historical" chapters on the development of the basic prokaryote genetic systems using the phages T4 and λ and the bacterium K12 as examples are of necessity only sketches of what took place and do not give a balanced idea of the nature of the discoveries involved or the problems which had to be overcome. More successful is the chapter on the properties of the less well-known phages which outlines clearly the amazing variation in the strategies which have been adopted in various situations, and there are two well-researched chapters on plasmids which bring together a lot of useful ideas and practical material. Transposition and its consequences are treated rather briefly, but there is a clear exposition at the end of the book of how modern techniques in gene manipulation are performed and of the new frontiers which are being opened up by these techniques.

Overall the book succeeds in what it sets out to do. It is clearly written, the diagrams and format are of a high standard, and it is as up to date as can be expected in such a rapidly expanding field. There is no doubt it will be a useful adjunct in teaching introductory courses in molecular genetics. □

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Dorothy A. Miller

Genetics: Human Aspects. By Arthur P. Mange and Elaine Johansen Mange. Pp.675. ISBN 0-03-056751-3. (Holt, Rinehart & Winston: 1980.) \$25.95, £14.95. *Human Chromosomes: Structure, Behavior, Effects.* By Eeva Therman. Pp.235. ISBN 0-387-90509-X. (Springer-Verlag: 1981.) DM37, \$21.80.

THE tone of *Genetics: Human Aspects* is set by the cover, which has a picture of Adam pondering as he sits alone on the stump of a primitive tree. Perhaps he is thinking about the fact that all human beings will arise from him and hence be similar, but never identical, to him. This is not to imply that this is a creationist text, but rather one which builds on the assumption that the study of human genetics should be of interest to a student just because he is a human being.

Mange and Mange have written a very readable book, indeed. It is aimed at those with only a moderate scientific background, not including a general genetics course. Hence the authors try to give a comprehensive presentation of genetics in which, whenever possible, concepts are illustrated by human rather than plant or animal examples. Simple statistics and chemistry are dealt with in appendices.

In addition to the easily understood, not to say sprightly, text, the book has much to recommend it. The material is up-to-date and relatively free of errors. The line drawings are particularly well conceived and are very effective in simplifying concepts as they are presented in the text. The references are well chosen and include some original articles so that the student can appreciate the step by step nature of research. The authors refer to numerous areas that still require research, leaving some room for student imagination. There are adequate questions at the end of each chapter and the answers are accompanied by explanations.

Will the book be suitable for a course? The fast pace that makes the book so enjoyable also means that most areas are not dealt with in depth. Hence, the text would be more suitable for undergraduate or adult education courses than for graduate or medical school courses. As in any text, the balance of material selected is a matter of personal preference which teachers must assess for themselves. They would be well advised to at least examine this text.

The material in *Human Chromosomes* by Eeva Therman overlaps about one-fourth of that in *Genetics: Human Aspects* because chromosomes play such an important role in human genetics. The text covers areas of human cytogenetics that have been studied using the light microscope: chromosome structure,

mitosis, meiosis, banding, structural and numerical abnormalities, changes in cancer cells and human gene mapping. Examples are drawn primarily from human material, but use has been made of plant or animal studies when these provide the best material available. The book does not deal with molecular approaches to cytogenetics and includes almost nothing about findings of biophysical, biochemical or electron microscopic studies (for example, nucleosomes are not mentioned).

Therman's writing is clear and her style didactic. She includes many definitions and presents the facts that have been established. Although she has carried out extensive research on the human X chromosome, her presentation is not research orientated. She does not, for example, discuss the way the facts were obtained, nor does she try to entice students into cytogenetics by emphasizing questions that remain to be answered. She prefers to cite reviews rather than original articles so that "the reader is encouraged to delve deeper into any question of interest"; whether this will have the desired effect is not clear. For me it produced a rather static image of an active field.



Cytogenetics is a visual science; unfortunately the illustrations of human chromosomes given in the book do not provide a very complete background for the student. Full human karyotypes are shown for unbanded Giemsa (two), G- and Q-banded cells, but not for C- or R-banded cells, which are represented by a partial karyotype of C-banded E group chromosomes and a single R-banded number 1. Reciprocal translocation is poorly illustrated by the karyotype of an unbanded endoreduplicated cell, even though the figure legend states the identity of the translocated chromosomes was confirmed with Q. This selection of illustrations must have been a matter of choice, rather than space limitation, because a full page is devoted to C-banding of plant chromosomes and another to a G-banded karyotype of the rat.

Human Chromosomes is an introductory text suitable for undergraduate or graduate students. Since the subject matter is not dealt with in great detail, it should be compared to presentations of the same material in general human genetics texts.

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Science or scholarship for students of developmental biology?

D.R. Newth

Patten's Foundations of Embryology, 4th Edn. By Bruce Carlson. Pp.672. ISBN 0-07-009875-1. (McGraw-Hill: 1981.) \$30.25, £16.25. *Development*, 2nd Edn. By Gerald Karp and N.J. Berrill. Pp.692. ISBN 0-07-033340-8. (McGraw-Hill: 1981.) \$32.95, £18.25. *Developmental Biology*. By Leon W. Browder. Pp.602. ISBN 0-03-056748-3. (Holt, Rinehart & Winston: 1980.) \$28.95, £13.95. *Introduction to Embryonic Development*. By Steven B. Oppenheimer. Pp.404. Hbk ISBN 0-205-06899-5; pbk ISBN 0-205-07348-4. (Allyn & Bacon: 1981.) Hbk \$23.95, £16.50; pbk £8.95.

THE student of developmental biology, or of its senior component animal embryology, does not lack for possible textbooks. However, perhaps paradoxically, only the most elementary can afford to be comprehensive, the more advanced being tempted to specialize in one of two ways. They may be restricted systematically, dealing for example, only with animals, only with vertebrates or only with *Xenopus*. Or they may be mechanism based and call widely upon prokaryote, unicellular, and plant and animal multicellular systems for illustrations of their themes. In either case one senses a certain discomfort in the authors and fears a lack of satisfaction in the student reader.

Of the four books now considered, one — Carlson's revision of Patten — is unashamedly selective both systematically and thematically. It derives from an earlier book which celebrated the developmental anatomy of chick, pig and man but had little use for other material. Dr Carlson has now remodelled the work with the object of narrowing the gap between accounts of formal changes in developing animals and their experimental analysis. His main changes have been to stress the morphogenesis of organs that have been important in analytical work, to give an account of limb development that is a genuine integration of the formal and the analytical, and to introduce experimental findings at points where they help to solve anatomical problems.

The material is presented clearly and the book is abundantly and effectively illustrated. The student will gain from the self-imposed limitation of the treatment in that at the end he or she will feel confident of, and in, what has been accomplished. Dr Carlson provides an introduction to laboratory studies on early chick development as a long appendix.

Karp and Berrill have produced a very much revised and improved second edition of a fairly ambitious text. They cover so much and their treatment is so good in so many ways, that it seems churlish to question the coherence of the picture of development that emerges. And yet, after the early chapters in which a deve-

lopmental story unfolding can be discerned, the book inevitably fragments into sections whose sequence seems arbitrary. This, I must emphasize, is not meant as a criticism only as an acknowledgement of the impossibility at the present time of ordering our presentation of developmental problems consistently.

Plants apart — and few texts successfully integrate them with animal material — the systematic coverage is catholic, and the emphasis biological. That is to say that the biochemical and molecular biological approaches do not predominate, although usefully presented at many points.

Karp and Berrill, in my view, offer one of the half-dozen best general texts of developmental biology now available in English. They will run to a third edition no doubt, and may then take the opportunity to improve the proof reading, most especially in correcting the spelling of proper names.

Browder's *Developmental Biology* is also a large and ambitiously conceived work, but leaning more heavily upon molecular biology, and being somewhat kinder to plants. It has the virtue of clarity and is well illustrated, but it too shows that selection of material has been a serious problem. It would not be simply an act of piety to include discussion of, say, the development of the immune system and of the origin of antibody diversity in a textbook of developmental biology, especially in one which concentrates on biochemical and cellular matters. Yet Browder skates over these and a number of other fields which might have been expected to attract him; amphibian metamorphosis, for example, is given

cavalier treatment.

Yet his discussions of the matters that he deals with are well balanced and should incite students to explore the original literature. Especially in the area of gene control of development, his accounts are among the best available at this level.

Oppenheimer's book is the shortest and claims only to be an introduction. It is eclectic in coverage, but not very kind to plants. It is written with enthusiasm, is sometimes a little careless, but deals with most of its topics in a stimulating way. However, as with so many elementary introductions, it does not avoid the danger of unquestioning acceptance of matters which are not quite as certain as all that. In a section quaintly headed "Gastrulation in Amphibian" (*sic*) is given a not very good account of the process in question, but with no suggestion that it may still have uncertainties or that it may vary from one amphibian species to another. Yet it does. Nor is bird gastrulation so simple, or so well understood, as the student reading Oppenheimer might believe.

These are, of course, complaints of a kind that most elementary texts invite. To avoid them is difficult, but the attempt must be made if the reader is to be helped into science rather than into scholarship.

The authors of all these books have clearly gone to some trouble with their illustrations. Many, especially the scanning electron micrographs, are excellent. But publishers will have to take greater pains to ensure that their production methods are equal to the challenge. □

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Biology: broad brush for a large canvas

E.C. Cox & R.M. May

A View of Life. By Salvador E. Luria Stephen J. Gould and Sam Singer. Pp.806. ISBN 0-8053-6648-2. (Benjamin/Cummings: 1981.) \$26.95, £10.95.

THIS introductory biology text aims to concentrate on "the great themes and arguments of biology", rather than to offer the encyclopaedic catalogue that characterizes many of its competitors. The result is an interesting and unusual text which in places has a minimalist air to it; this is particularly true in the earlier sections, where a lot of the material is simply set forth without critical examination of the historical or contemporary experiments that compel belief.

The broad pattern of organization is the conventional one: beginning with the basic

chemistry of living things, we progress through genetics, developmental biology and the functioning of organisms to evolutionary biology and ecology. The presentation is uniformly lucid, easy to read and backed by first-rate line drawings and other illustrations (along with "boxes" developing special points).

Among the early chapters are some strikingly elegant and careful discussions: the structure of water; allosteric effectors (where the models and figures are superb); introductory genetics and gene regulation in bacteria. The molecular biology is not as contemporary as one might wish, virtually passing over much of the exciting new work on the gene structure of higher eukaryotes (such as gene inserts, the splicing together of variable and constant regions of the

globulin chains, the molecular changes associated with the regulation of mating type in yeast and so on). In short, the early sections are strong on the classic things, but appear a bit dated.

The chapters on the functioning of organisms do a good job of covering the major problems faced and solved by higher organisms: nutrition and gas exchange, transport, hormone functioning and the like. The emphasis is strongly on human biology (again with excellent illustrations), with occasional genuflection toward comparative and evolutionary analyses.

The concluding chapters focus on the broad themes of evolutionary thinking: the history of the subject, micro- and macro-evolution, biogeography and the fossil record. No other introductory text treats this material so thoroughly or so well. These chapters expound the essential philosophical character of Darwin's theory, and discuss problems associated with it, both past (blending inheritance; Mivart's doubts about the evolution of the incipient stages of useful structures, as instanced by the two reptilian jaw bones that are ear bones in contemporary mammals) and present ("neutral evolution"; mechanisms that can maintain heterozygosity in populations; cladist, phenetic and classical evolutionary schools of taxonomy). Given the current misrepresentation of evolutionary thinking by the so-called "scientific" creationists, these chapters are especially valuable. They are marred only by an occasional tendency to intrusive editorial comment, and by the misrepresentation of Darwin as holding that evolution proceeds at a consistently gradual rate, in contrast with contemporary notions about "punctuated equilibrium". As observed by Andrew Huxley in his Presidential Address to the Royal Society last year, Darwin's statement that "each form remains for long periods unaltered, and then again undergoes modification" and

... the periods during which species have been undergoing modification, though very long as measured by years, have probably been short in comparison with the periods during which these same species remained without undergoing any change ...

effectively makes him a punctuated equilibrist.

The treatment of population genetics is brief and not well organized, and that of population biology and ecology is perfunctory.

In short, this is a distinctive introductory biology text that all teachers of such courses will profit from reading. But we feel it is a bit too thin and quirky to displace the canonical Curtis or Keeton, unless supplemented by extensive additional readings. □

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The multifarious questions of evolution

Bentley Glass

Understanding Evolution. By Earl D. Hanson. Pp.556. ISBN 0-19-502784-1. (Oxford University Press: 1981.) £14.95, \$23.95.

THE recent flurry of attacks on school textbooks and the teachers of biology who deal with evolution should focus attention on the college textbooks of evolution and the way in which the subject is taught. The questions to be asked are multifarious. Is organic evolution itself, as distinct from the mechanism of natural selection, a theory or a fact? Is adequate attention given to the gradual change of ideas about a theory, starting in the case of evolution with the first glimmerings of the concept among the Greek philosophers and the ancient and mediaeval belief in spontaneous generation? What of the long debate over the inheritance of acquired characteristics, continuing even today? Just what was Darwin's contribution to the theory of evolution? How does the evolutionary synthesis of today differ from Darwin's own ideas? There are many more.

In most respects, the teacher — if not the student — will welcome Hanson's book. It is comprehensive and clearly written. As might be expected from his earlier books, Hanson emphasizes animal and plant diversity, homologies and relationships. But there is a substantial section devoted to Mendelian and modern genetics as the basis of understanding what Darwin always affirmed was the crux of the matter: the nature and origin of hereditary variations. There is also a detailed treatment of the evidence for relationships that comes from biochemical comparisons of amino acid sequences in proteins and nucleotide sequences in DNA. For a course intended to run only for one semester of a college year, there may be far more technical detail here than a student can master; yet better too much than too skimpy a treatment.

As for noticeable deficiencies in the book, it seems to me that not enough attention is given to the history of the idea of evolution, to the pre-Darwinian as well as post-Darwinian gropings for an explanation of origins, to the marvellous combination of evidence from every sub-field of biology. I notice also a tendency to deal rather briefly with the evolution of plants, although their speciation by polyploidy, especially allopolyploidy, affords some of the best evidence for the origin of species in recent times. The coverage of plant and animal geography in relation to evolution is also somewhat meagre. Nevertheless, the classical evolutionary synthesis is well expounded.

Finally, in respect to human evolution, in my opinion a fuller development of primate and hominid evolution would be enjoyed by teacher and students alike. It is

difficult, in this area of knowledge, to keep pace with discovery and choose between conflicting interpretations. Hanson has relied heavily on the work of the Leakeys. He omits entirely any reference to the breathtaking discovery of "Lucy", the three-million-year-old, erect-walking australopithecine whose complete skeleton is the most magnificent and astounding of all palaeoanthropological discoveries. This section of Hanson's book already cries out for revision.

The last 200 pages of *Understanding Evolution* are devoted to a phylogenetic survey of plant and animal groups. That is clearly the author's own bent. Other teachers may wish that those pages had been used for a fuller treatment of some of the matters I have mentioned that have received little attention. In particular, there would be a solid advantage in allotting space to an explicit exposé of the assumptions and subterfuges of the "scientific" creationists. □

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Natural history of vertebrates

Barry Cox

Patterns of Vertebrate Biology. By E.W. Jameson, Jr. Pp.477. ISBN 0-387-90520-0. (Springer-Verlag: 1981.) DM 63, \$33.20.

THE preface to Jameson's book states that it is concerned with the natural history of vertebrates, and he comments that "the laboratory, however essential and productive, is a departure from the animal's real world". Much of the book is therefore primarily descriptive, made up of a collection of discussions of different aspects of the field biology of vertebrates.

However, Jameson commences with chapters concerned with "An Overview of Vertebrate Phylogeny", "Mechanics of Evolution" and "Zoogeography". It is always difficult to know where to draw the line between relevant topics and those which are merely general background, but I thought that none of these contributed significantly to Jameson's main thesis, while the first also revealed an uncertain knowledge and understanding of this topic.

The remainder of the book is divided into three parts: "Individual Environ-

mental Responses" (with chapters on breathing, food and feeding, thermoregulation and water balance, activity and seasonal dormancy); "Communication" (sensory receptors and perception, signals); and "Population Phenomena" (reproduction, growth, community and population density). Here again, there are sections that have little to do with natural history. For example, the details of the structure and ventilation of the bird's lung and airsac system are merely functional anatomy, and only become natural history if comparative data are given on variations in the structure or relative size of the system

in different birds, such as penguins, ostriches, humming birds and so on. The book is also not without errors: for example, hagfish do not have only a single pair of gills (p.104), and the spiracle is not the vestigial last gill (p.106).

Nevertheless, those wishing to flesh out structural anatomy with interesting examples of natural history will find many useful examples in this book, and there is an extensive bibliography of well over 1,000 entries. □

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The welcome return of J.Z. Young

L. Beverly Halstead

The Life of Vertebrates, 3rd Edn. By J.Z. Young. Pp.645. Hbk ISBN 0-19-857172-0; pbk ISBN 0-19-857173-9. (Oxford University Press: 1981.) Hbk £25, \$26.95; pbk £14. *The Chordates*, 2nd Edn. By R. McNeill Alexander. Pp.510. Hbk ISBN 0-521-23658-4; pbk ISBN 0-521-28141-5. (Cambridge University Press: 1981.) Hbk £35, \$69.50; pbk £12.50, \$24.95. *Lecture Notes on Vertebrate Zoology*. By R. Pearson and J.N. Ball. Pp.180. Pbk ISBN 0-632-00729-X. (Blackwell Scientific/Halsted: 1981.) £7.50, \$21.95. *Anatomy of the Chordates*, 4th Edn. By C.K. Weichert. Pp.814. ISBN 0-07-069007-3. (McGraw-Hill: 1981.) \$37.75, £20.95.

In 1950 J.Z. Young published *The Life of Vertebrates*, which rapidly came to dominate the teaching of vertebrate zoology at a time when the vertebrates occupied an entire academic year in most zoology degree courses. In 1962 the second edition appeared, but by the end of the 1960s and throughout the 1970s there was no J.Z. and teachers were obliged to look elsewhere. Not only that, but vertebrate zoology had to relinquish much of its territory as newer aspects of biology rose to the fore. Vertebrate courses became a catalogue of anatomy, with long names to be studied and learnt for no other apparent reason than that it was supposed to do students good. The vertebrates tended to be relegated to a smaller and smaller part of the curriculum. As this process was underway, by some curious irony advanced texts, monographs and symposium volumes on the group seemed to be constantly pouring out of publishing houses; yet there was nothing remotely suitable in the way of a general textbook.

In 1975 there appeared McNeill Alexander's *The Chordates*, a general textbook of vertebrate zoology written by a leading expert in biomechanics, which in many ways fitted the bill. Here, the structures of the vertebrates were discussed from the standpoint of their mechanics.

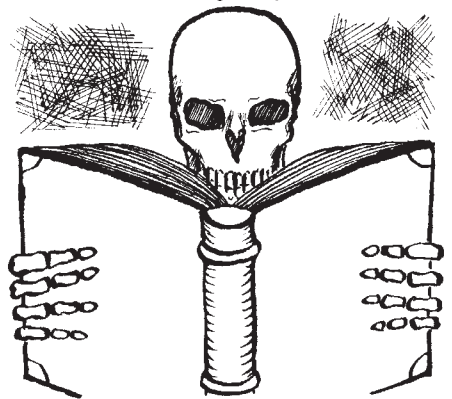
This approach lent itself especially to practical demonstration of the basic principles involved and was a truly excellent contribution to teaching, though the mathematical aspects tended to intimidate both the students and the less numerate teachers. However, this textbook signalled a refreshing new approach that deserved encouragement. The publication of a second edition, which has been updated and includes several new topics such as a useful discussion of dinosaur biomechanics, augurs well for the future of vertebrate zoology and the biomechanical approach being promoted by Alexander.

The appearance of Pearson and Ball's *Lecture Notes on Vertebrate Zoology* clearly fills a need in the teaching scheme of things. It is sad that it should be so. This book as far as one can judge sets out to record all the boring bits of the subject, with its emphasis on pure anatomy. The authors' aim "to release the teacher from the drudgery of simply reproducing anatomy and to create a platform for profitable expansion on innumerable other topics". Well, here is the drudgery all right but I fear that having created this platform, far from inspiring a prospective student into "profitable expansion" it would convince him that the subject is one of stupefying boredom to be avoided at all cost. The illustrations are notable for their variable quality: the crude sketch of the structure of a reptile scale (p.93) conveys nothing to me, and I suspect the authors would not have included it had they ever looked at a microscopic section of, say, lizard skin. Worse, some of the information is so out of date that it would appear that the authors have cobbled together their material from a selection of older textbooks. I suppose a teacher could use the book to instruct students in some basic information but they would be failing in their duty to allow students a glimpse of it. This book can only do harm in that it is guaranteed to snuff out any spark of

enthusiasm.

Last year also saw the appearance of the fourth edition of Weichert's *Anatomy of the Chordates*, a system by system comparison of the chordates — a worthy, albeit rather fusty book, again dating from a time (1951) when the vertebrates formed a larger proportion of the syllabus than today. This book, however, can be recommended for all teachers of vertebrate zoology in that it is relatively easy to pick out how, for instance, the vascular system varies from one major structural grade of vertebrate to another. This is the kind of book to have to hand as a reference for teaching.

For teachers and students of vertebrate zoology the publishing event of 1981 was, however, the long awaited return of J.Z. Young, with the third edition of *The Life of Vertebrates*. It all comes back now, why J.Z. had such a profound effect on both teachers and students; here he speaks directly to the reader about really fascinating topics. It is always possible to complain that the choice of topics is rather too personal but this is surely what gives the book its vitality. The notable thing about *The Life of Vertebrates* is that a mass of information is communicated in such a light and easy manner; but, more important, the enthusiasm of youth pervades this book. There are odd bits where one cannot help but be irritated because it is either grossly out of date or



plain wrong, such as the hopelessly inaccurate diagram of the visceral arches and jaws in early vertebrates. One of the greatest surprises is that the current controversies on methods of classification and the arguments over the process of evolution and the fossil record are alluded to and the clear voice of common-sense always characterizes J.Z.'s overview, not only of current fashions but of the place of zoology in its wider perspective.

The pre-eminent position of J.Z. Young as the teacher and enthusiast of the study of the vertebrates is richly deserved. This is the best textbook around. The occasional lapses can be forgiven; it is the inspiration that he provides that will encourage a new generation to come into vertebrate zoology. □

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No bones, but plenty of meat

A.E. Brafield

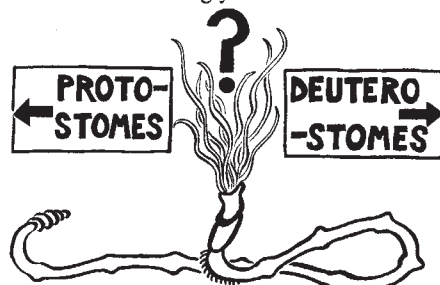
Invertebrate Zoology, 4th Edn. By R.D. Barnes. Pp.1,089. Hbk ISBN 0-03-056-747-5; pbk ISBN 4-8337-001-8. (Holt-Saunders: 1980.) Hbk £17.95, \$27.95; pbk £11.50. *Invertebrate Zoology*, 3rd Edn. By J.G. Engemann and R.W. Hegner. Pp.746. ISBN 0-02-333780-X. (Collier Macmillan/Macmillan, New York: 1981.) £13.95, \$20.95. *Invertebrate Respiration*. By R.M.G. Wells. Pp.72. Pbk ISBN 0-7131-2806-2. (Edward Arnold: 1980.) £2.60. *Practical Invertebrate Zoology*, 2nd Edn. Edited by R.P. Dales. Pp.356. Pbk ISBN 0-632-00755-9. (Blackwell Scientific/Halsted: 1981.) £9.50, \$32.95.

A SYSTEMATIC and detailed study of the vast array of invertebrate animals continues to form a substantial part of undergraduate teaching in zoology. The problem of striking a satisfactory balance between their anatomy, physiology, ecology and evolutionary relationships has become more acute as our knowledge in these areas has increased. A sound and comprehensive textbook to supplement "the invertebrate course" is essential, and two which have built up a considerable reputation have recently appeared in new editions.

Invertebrate Zoology, by Barnes, has reached its fourth edition. Barnes claims that "there is no way a book of this sort can avoid growing to some extent", which is debatable to say the least, but most of the considerable increase in length of this edition comes from the many new drawings and photographs. The new artwork by Mary Ann Nelson is excellent and she has made her drawings from actual specimens (a habit which seems, unfortunately, all too rare). The text is as clear and sound as ever. Summaries have been added, a central section of over 40 colour photographs is a new feature, and there is now an interesting chapter on coral reefs. Barnes still plays down the insects too much, however, allowing them only about 30 pages in a book of over a thousand. The paperback version has a much smaller page size than the hardback and the consequent reduction in the size of typeface and diagrams tends to make the text hard to read and the illustrations less clear, but the paperback, being roughly half the bulk and weight of the hardback, is certainly easier to handle.

The third edition of *Invertebrate Zoology* by Engemann and Hegner is also welcome. (Engemann's name now precedes that of the late Robert Hegner.) It is as concise and well organized as previous editions, with key words in italics and the material arranged in short, headed sections. Engemann is a devotee of the "type approach", in which one species is treated in great detail to serve as an example of its group. A drawback of this

practice, it seems to me, is that every aspect of the selected type is described, whether it happens to be typical of the group or not, at the expense of more information on other members. (The crayfish occupies as many pages as all other crustaceans put together, for example.) An unusual feature is the new chapter on deep-sea invertebrates, in which the suggestion that these animals tend to be extremely long-lived is critically examined in order to discuss "a broad range of principles" of invertebrate zoology and to encourage the student to think more deeply. A worthy aim, which Engemann achieves convincingly.



The division of coelomates into protostomes and deuterostomes is coming under some attack nowadays, but it is a convenient and useful concept and both Barnes and Engemann retain it. They disagree, however, over many points of classification. For example, Barnes puts the pogonophorans among the protostomes, rightly I think, whereas Engemann still lists them among his deuterostome groups (although he acknowledges elsewhere that their position is puzzling). Engemann discusses the problems of protozoan relationships but in the end sticks to the system customarily used by zoologists, whereas Barnes now raises each of the major protozoan groups to the rank of phylum (in the Kingdom Protista). Such disagreements over classification are inevitable, but they can confuse students. Barnes has dropped his earlier chapter on phylogeny, and Engemann also avoids, sensibly, a long consideration of possible evolutionary relationships between the phyla.

In addition to comprehensive textbooks, there is now a wide variety of smaller works which cover specific aspects of invertebrate zoology. *Invertebrate Respiration*, by Wells, is a fine example. The author's lively style reflects his great enthusiasm for the subject, and the longest chapter deals effectively with the comparative physiology of blood pigments, Wells's own speciality. Zoologists with no great feeling for the physical sciences will find the introductory chapter on the principles of gaseous exchange particularly valuable.

Laboratory classes are an indispensable part of invertebrate courses. They generally include an intensive study of anatomy (by microscopy and by

dissection), demonstrations of specimens to illustrate the range of form within each group, and some simple experiments. All these aspects are well served by the second edition of *Practical Invertebrate Zoology*, edited by Dales. He and the six other authors are all experienced university teachers and each is an expert on one of the major invertebrate phyla. The book is well written and the material sensibly arranged. There is too much writing and not enough illustration for a laboratory manual, to my mind, but the book contains a great deal of valuable information. The section on general methods, and the numerous suggestions for simple experiments, make the work a useful sourcebook for the teacher as well as the student. □

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Insects entomosed

M.E.G. Evans

The Science of Entomology, 2nd Edn. By William S. Romoser. Pp.575. ISBN 0-02-403410-X. (Collier Macmillan/Macmillan, New York: 1981.) £13.95, \$21.95. *Fundamentals of Entomology*, 2nd Edn. By Richard J. Elzinga. Pp.422. ISBN 0-13-338194-3. (Prentice-Hall: 1981.) \$22.95, £17.20. *Insect Physiology*. By W. Mordue *et al.* Pp.108. Pbk ISBN 0-632-00385-5. (Blackwell Scientific/Halsted: 1981.) £5.75, \$16.95.

ENTOMOLOGY is one of the few component parts of zoology to be taught as a science in its own right. Physiology, parasitology and cytology, for example, are major topics covering the whole of the animal kingdom; the Insecta and Chordata are the only individual groups to merit major topic treatment, and this is reflected by the large number of books concerned with their study. One might wonder whether more entomology texts are really necessary — perhaps merely to include recent advances? — but since the fundamentals of insect biology do not change, a sensible solution is to rewrite the better books. Two of the three texts reviewed here are second editions of books already established in North America. All three are good examples of the recent generation of entomological texts which pack a large amount of information into a single, readable and well-illustrated volume. Modern textbooks are not necessarily better than some of their predecessors, but they are often more lucid and are usually more attractive visually; studying is still hard work, but now it may be less painful.

Romoser and Elzinga have each written comprehensive accounts which emphasize the general biology of insects rather than

their classification or economic importance. *The Science of Entomology* is the larger book and is clearly intended for intermediate or advanced courses (second or third years in Britain; for North America, the author suggests a one-semester course). It has a rather academic approach of the sort found in a good standard text such as Richards and Davies's *Imms' General Textbook of Entomology* (10th Edn, Wiley/Chapman & Hall; 1977). Romoser ranges from morphology and physiology to ecology and evolution, allotting a chapter each to applied entomology and systematics. For completeness, an entomology course would need a more extended treatment of systematics based on a text such as Richards and Davies or Borror, DeLong and Triplehorn's *An Introduction to the Study of Insects* (5th Edn, Holt, Rinehart & Winston; 1981). In fact, such back-up is emphasized throughout the book, as demonstrated by the introductory section on entomological literature and by the impressive number of references cited.

Fundamentals of Entomology is an introductory-course book which aims to cover the whole of entomology at a more elementary level. Again, it is based on general biology, but there is also a lengthy classificatory section with keys to both orders and major families. It is profusely illustrated with line drawings and photographs; however, as in many zoological texts, the macrophotographs sometimes lack the clarity of the scanning electron micrographs and the precision of the line drawings. Most of the topics have been treated by Elzinga in a compact and readable manner, although it might have been wiser to omit some sections rather than over-reduce them; for instance, it is difficult to explain population dynamics in eight pages.

Insect Physiology is a multi-author paperback which deals with only part of a large subject. It is a clear exposition of homeostatic problems — from cell metabolism to nerves and muscles — although only about one-fifth of the book deals with behavioural physiology. In spite of four authors, the chapters link together well and the diagrams are excellent. A few key references are quoted at the end of each chapter to guide the student to the more important literature; I would have preferred a few more in order to reach the most interesting topics more rapidly. Although an introductory text, the book has the merit of conveying some of the fascination of recent research in this field.

All three books are welcome additions to the field of insect biology. Elzinga can be recommended for a short, introductory course, Romoser for a major part of a longer, more advanced course, and Mordue for the up-to-date physiology section of a general entomology course.

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Saline solutions

D.J. Crisp

Elements of Marine Ecology: An Introductory Course, 3rd Edn. By R.V. Tait. Pp.356. ISBN 0-408-71054-3. (Butterworths: 1981.) £8.95, \$22.50. *The Estuarine Ecosystem*. By Donald McLusky. Pp.150. Hbk ISBN 0-216-91115-X; pbk ISBN 0-216-91116-8. (Blackie/Wiley: 1981.) Hbk £12.75, \$29.95; pbk £6.25. *The Ecology of Marine Sediments: An Introduction to the Structure and Function of Benthic Communities*. By John S. Gray. Pp.185. Hbk ISBN 0-521-23553-7; pbk ISBN 0-521-28027-3. (Cambridge University Press: 1981.) Hbk £15, \$34.50; pbk £6.95, \$16.50.

AN ELEMENTARY text should not only provide a clear and comprehensive account of its subject, but it should also stress those areas where there is a consensus of view and give less prominence to more controversial subjects where current research has yet to resolve the pattern. In doing so it will provide the reader with an historical perspective, identify the founders of classical works and provide up-to-date reviews.

The third edition of Tate's *Elements of Marine Ecology* achieves most of these objectives. It differs little in content from earlier editions and fully meets the needs of elementary students of marine biology who seek only the minimum of understanding of chemical and physical oceanography. The reference lists have been considerably enlarged and the division into books for general reading and specific references to textual matters has been eliminated. Neither of these changes seems particularly beneficial, other than to a reference collector. The majority of classical marine biology works are included but so also are a frightening number of minor papers. The author therefore has done less than he might in guiding the student towards serious and essential reading. Nonetheless, it remains a sound, well-ordered and very useful student text. There are no obvious errors, except for the blatant misuse of the word "parameter" in the title of Chapter 4 — alas, a fault now almost sanctioned by usage.

The Estuarine Ecosystem is aimed at a more specialized readership. The first chapter includes some elementary consideration of the hydrography, sedimentary processes, species composition and food webs of estuaries. It is a rather superficial account suffering from false simplification, perhaps on the wrong assumption that students of marine biology should not have to bite on hard science. To take a simple example, sinking rates are discussed in relation to the impact law for large particles and Stokes' law for small ones, but the underlying reason behind the different approaches is not explained. Neither the bounds over which the equations hold true nor their

derivations are given, yet this information is exactly what the intelligent student needs in order to grasp the relevant scientific principles. In the same chapter, hydrographic features of estuaries are not fully discussed in terms of estuary size and salinity fluctuation, while recent work on the importance of rate of change of estuarine variables on the tolerance of animal species is not mentioned. Indeed the diagrams showing species penetration into estuaries are related to "salinity", not even "mean salinity" and certainly without reference to fluctuation.

Insufficient consideration is given to estuarine topography especially the influence of coastal emergence and submergence on estuarine form. The book as a whole deals almost exclusively with British and eastern American coasts, omitting any consideration of Arctic and tropical estuaries. In regard to the historical perspective of the subject, it fails dismally. The names of Bowden, Ketchum and Redfield, and Riley never appear, while in the place of the original figure depicting the mid-estuarine minimum in faunal diversity, given by Alexander *et al.* in their classical paper of 1935, the author quotes only from his own book of 1971.

The book includes a useful accumulation of productivity data for estuaries, with many food web diagrams, but little of novelty or abstracted wisdom which cannot be found elsewhere. The reference lists do not appear to be selective or exhaustive.

John Gray's *Ecology of Marine Sediments*, though less weighty in information, contains the essential material for the author's purpose and a good historical background. It aims to focus the minds of benthic ecologists on the interpretation of data in terms of modern ecological principles. Gray seizes on the linearity of the log-normal distribution of individual numbers in relation to species as a good measure of community equilibrium in the absence of disturbance. One would like to have seen a further extension of the philosophical basis of the idea, with a guess at the ecological meaning of the relevant statistics.

The book contains thoughtful discussions on niches, diversity and faunal stability — ideas that are currently being tested by ecologists in many environments. Finally, the author considers practical means of monitoring benthic communities in respect of long-term effects of pollution or of other factors that may control their structure.

This volume affords good reading for the above-average student of marine ecology who is prepared to sit and reflect, and is essential for those engaged in research on the fauna of sediments. It is a valuable contribution to ecological theory.

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Aspects of environmental physiology

J.A. Riegel

Functional Adaptations of Marine Organisms. Edited by F. John Vernberg and Winona B. Vernberg. Pp.347. ISBN 0-12-718280-2. (Academic: 1981.) \$39.50, £26.20. *Animal Osmoregulation.* By J.C. Rankin and J. Davenport. Pp.202. Hbk ISBN 0-216-91014-5; pbk ISBN 0-216-91015-3. (Blackie/Wiley: 1981.) Hbk £17.95, \$39.95; pbk £8.95.

THESE two books have one common feature: they represent attempts to relate various aspects of organismal function to environmental variables. That edited by the Vernbergs is a multi-authored treatise whose main use will be as a source of reference. Rankin and Davenport's book is much more didactic in its approach, and it should find a wide audience amongst students and teachers of courses in environmental physiology.

Functional Adaptations of Marine Organisms contains chapters written by specialists on primary production, bacteria, zooplankton, meiofauna, benthic macrofauna, pelagic macrofauna and deep-sea organisms. In addition, the editors have provided an introductory chapter describing the ecological subdivisions of the marine habitat. This is both welcome and necessary for the non-specialist reader. Like most multi-authored works, however, the book's coverage is inadequate. For example, the discussion of zooplankton is confined to copepods, and primary producers are reviewed largely from an ecological viewpoint. Recent discoveries of communities of animals densely clustered around deep-sea, warm-water vents and dependent upon the primary production of chemosynthetic bacteria receive only brief mention. Nevertheless, the major shortcoming of the book is due to the inadequacy of the background data. Although more could have been done to round out the material, this deficiency is not altogether the authors' (or editors') fault — precious little is really known about the functional adaptations of marine organisms, especially those in the less-accessible parts of the sea.

Despite its shortcomings, the book should be a useful reference work for specialists and non-specialists alike. Although no chapter represents an exhaustive review of the literature, most authors have included references to reviews and papers covering aspects of their subjects not discussed extensively in their contributions.

Animal Osmoregulation presents a refreshingly uncluttered overview of not only the basic concepts of the subject, but also most of the currently-active fields of research. The book starts with a consideration of basic principles, the explanations of which should be of sufficient clarity to be understood readily by the average under-

graduate. Following this come discussions of the adaptations of animals to various habitats, from the sea to brackish water, fresh water and land. Specialized adaptations of osmoregulatory mechanisms, such as the mammalian kidney, buoyancy and to life in acid and alkaline waters, are also covered. The examples used to illustrate the various adaptations are highly selective and several are drawn from the research of the authors and their associates. Perhaps it is this personal experience of much of the research underlying the material which gives the narrative an admirable lack of dogmatism. Aware of the highly-variable nature of most data, they tend to generalize with caution.

The final chapter of *Animal Osmoregulation* deals with some of the common physiological and behavioural techniques used in studies of osmoregulation. The description of physiological techniques may provide useful background, but the short discussion of behavioural techniques should prove more valuable, especially as the basis of classroom experiments or demonstrations. □

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Adaptation to supply and demand

Garth Chapman

Animal Physiology: Adaptations in Function. By F. Reed Hainsworth. Pp.669. ISBN 0-201-03401-8. (Addison-Wesley: 1981.) \$25.95, £10.95.

HAINSWORTH'S *Animal Physiology* declares itself to be ecological and comparative by its subtitle but only discloses the fact that it is "organized around" an "economic theme for comparative physiology" in the preface. The animal's balance sheet of benefits and costs uses the currency of resources and concludes about joules and survival what Mr Micawber concluded about pence and happiness.

This is certainly an important theme but it becomes a little tedious by reiteration. However heuristically valuable such an approach may be when applied to resources such as oxygen, food, water and salts, it is less directly applicable to that large and distinctive part of animal physiology which is concerned with reception, co-ordination and response. Accordingly, perhaps, the section dealing with these topics is placed at

the end of the book. Although not neglected, less prominence is given to them and to the current tenets of membrane physiology on which they are based than in some recent texts such as Goldstein's *Introduction to Comparative Physiology* (Holt, Rinehart & Winston, 1977) and Eckert and Randall's *Animal Physiology* (W.H. Freeman, 1978) where they follow the opening chapters on physical and chemical principles.

In style the book is densely and didactically written but the terse generalization is usually supported by example or the brief report of an observation or experiment. Nevertheless, I found myself wondering whether an introductory text ought not to lead readers more gently along the path of interest rather than seek to impress them with sheer quantity of information. Unlike some bulky and extravagant textbooks, this one

is handy and well produced apart from a few gimmicks such as two contents lists, one short and obscure and one detailed and eleven pages in length! There is no indication of part or section in the running headlines, which makes cross-reference difficult since such references are frequently indicated by subdivision and not by page. Bibliographical references (not indicated in the text) are divided into annotated, at the end of each part, and "credit" — whatever they may be — at the end of the volume.

In short, this seems a somewhat daunting introductory text to read although good indexing increases its value to an industrious student for purposes of reference and study. □

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Enzymology: the general and the specific

R.N. Perham

Understanding Enzymes. By Trevor Palmer. Pp.405. Hbk ISBN 0-85312-202-4; pbk ISBN 0-85312-307-1. (Ellis Horwood, Chichester/Halsted: 1981.) Hbk £25, \$80.95; pbk £9.50. *Molecular Enzymology.* By Christopher W. Wharton and Robert Eisenthal. Pp.326. ISBN 0-216-91012-9. (Blackie/Wiley: 1981.) £19.75, \$46.95. *Isoenzymes.* By C.C. Rider and C.B. Taylor. Pp.78. Pbk ISBN 0-421-15640-7. (Chapman & Hall/Methuen: 1981.) £2.45, \$5.95. *Enzyme Kinetics: The Steady-state Approach*, 2nd Edn. By P.C. Engel. Pp.96. Pbk ISBN 0-412-23970-1. (Chapman & Hall/Methuen: 1981.) £2.45, \$5.95.

THERE is hardly a corner of biochemistry or molecular biology — not to mention the cognate biological and chemical sciences — where modern ideas of enzymology have not intruded to good effect. The study of enzymes is therefore properly an important part of all degree courses in these subjects, and there continues to be no shortage of authors willing to try their hand at providing the necessary textbooks.

Dr Palmer's offering, *Understanding Enzymes*, attempts to cover all of the fascinating aspects of enzymes, from their biosynthesis to their structure, chemical mechanisms and kinetics, from their place in metabolism to their use in medicine and industry. It is an heroic task but one of dubious practicability — witness how few authors have followed the trail so comprehensively blazed by Dixon and Webb in their classic first edition of *Enzymes*, published by Longman. Thus one should not be surprised that in the volume under review, which is furthermore written by a single author, the coverage is uneven and appears to rely heavily on secondary

sources. It is sad to record that some of those sources are significantly out of date. For example, in the short chapter on protein structure nothing published since 1970 is suggested for further reading, the chapter on biosynthesis of proteins (surely now misplaced in a book on enzymes) has no specialist reference later than 1974, and the chapter on metabolic regulation includes a diagram of the cell that places the cisternae of the rough endoplasmic reticulum in unrestricted communication with the extracellular environment. The kinetics of enzymes are dealt with more extensively and sympathetically, and the later sections on technological and medical applications of enzymology cover a wide range of topics. Each of the 20 chapters concludes with a useful summary of its contents and most carry a set of problems and answers. In what I take to be an otherwise commendable attempt to lower costs, many of the illustrations, though clear, appear to have been left as sketches, which adds a slightly makeshift air to the printed page.

All in all, I cannot see this book supplanting the more narrowly based texts on which the undergraduate teaching of enzymology normally rests, though the convenience of a broad range of topics falling within a single volume may commend it to those with less stringent needs.

A new contender for a place among the more specialized texts is *Molecular Enzymology* by Wharton and Eisenthal, which assumes a basic knowledge of university biochemistry and chemistry. The approach is first to tackle the chemical aspects of catalysis and the properties of enzymes as proteins, to develop ideas in

enzyme kinetics and then to deal in some depth with the mechanisms of eight or nine selected enzymes as examples. The book concludes with a return to the practical problems of studying enzyme kinetics in the laboratory. The treatment is clear and conventional, cuts few corners and makes plain the complementarity of the different methods; it should appeal particularly to those with a background in the physical sciences. The bibliography is sound and extensive. The authors eschew the usual efforts to represent three-dimensional events on paper in favour of a more descriptive turn-by-turn account of the involvement of amino acid side-chains and other functional groups, an approach with which I do not altogether agree. And at the risk of a charge of chauvinism, I suggest that any new text on molecular enzymology that largely neglects protein mobility and dynamics, multienzyme systems and multifunctional proteins cannot be regarded as complete. These quibbles apart, I found little to quarrel with and expect the book to find an honourable place in university reading lists.

Finally, it is a pleasure to notice two further additions to the series of *Outline Studies in Biology*. The first, *Enzyme Kinetics* by Engel, is the second edition of his successful account of steady-state kinetics and their contributions to problem-solving in enzymology. The second, *Isoenzymes* by Rider and Taylor, describes the importance of isoenzymes in a broad range of biochemical and medical contexts. Both are clear, unpretentious and informative, in accord with the general style of this well-known series. □

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Spanning a bridge

Peter H.W. Butterworth

Biochemistry, 2nd Edn. By Lubert Stryer. Pp.949. Hbk ISBN 0-7167-1226-1; pbk ISBN 0-7167-1306-3. (W.H. Freeman: 1981.) Hbk \$32.95, £24.40; pbk £12.95.

THERE are a lot of biochemistry textbooks and, as the borders of the subject continue to be extended, there will be more. Some adopt a particular emphasis; others attempt a comprehensive approach to the subject. Lubert Stryer's text belongs to the latter genre and it is doubtful whether there will be another which will appeal to the student as strongly as this one does. When the first edition appeared, immediate reaction was dominated by the impact of the remarkable illustrations and the succinct text. Familiarity with the book brings with it the realization of how

ingeniously interdependent these facets are: the illustrations are not just attractive but they are required and used to support the concise style of the text. At the same time, the student is provided with a graphic framework into which complex concepts often seem to sit with comfort.

Every author of a basic biochemistry textbook is faced with two decisions: first, at what level to pitch the book and second where to begin and end. Stryer seems to aim unashamedly for the "early undergraduate", yet the brevity of the text often belies its depth: the author has a unique ability to expound difficult concepts in a few words. As biochemistry constitutes a bridge between physical and biological sciences, the limits are difficult to define and, regardless of how far apart the covers are, it would be impossible to satisfy every whim. Here the subject matter is very broadly based and the student is required to accumulate background knowledge of the physical sciences, organic chemistry, cell biology and basic molecular genetics from elsewhere. Nevertheless, one of the strengths of this textbook is that it can be

appreciated before the background is complete.

It is not surprising that this second edition is bigger than the first. Neither is it surprising that many of the illustrations in the first edition have been updated and many more have been added; a major effort has been made to get across the concept of the integration of metabolic processes and the text has been extended greatly to accommodate major advances in the subject, particularly in the area of molecular biology.

Textbooks for major subjects often present a daunting prospect where they should foster the enthusiasm of the student and provide a realistic challenge. The response to Stryer is generally enthusiastic: for a textbook, that is an accolade. The only penalties for confronting so massive a task may be that the compact style sometimes excludes subtlety and may not leave room for mystery, the solving of which will provide the material for the next edition. □

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Whole chemistry?

Peter Garratt

Chemistry: An Introduction to General, Organic, and Biological Chemistry. By Joanne M. Widom and Stuart J. Edelstein. Pp.799. ISBN 0-7167-1224-5. (W.H. Freeman: 1981.) \$23.95, £16.60. *Atoms, Molecules, and Life: An Introduction to General, Organic, and Biological Chemistry.* By Michael S. Matta and Antony C. Wilbraham. Pp.721. ISBN 0-8053-9640-3. (Benjamin/Cummings: 1981.) \$22.95, £14.95. *Basic Chemistry of Life.* By Milton Toporek. Pp.521. Pbk ISBN 0-8016-5002-X. (C.V. Mosby: 1981.) \$7.95, £12.

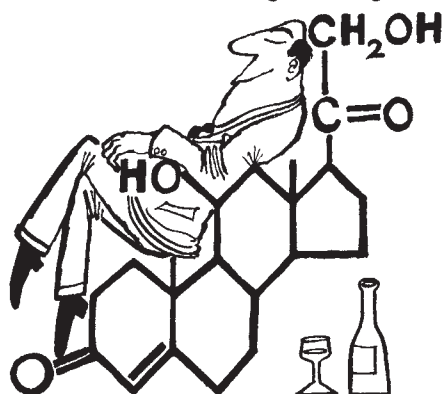
THESE three texts are all directed at North American students of the life sciences and each presupposes little previous exposure to chemistry. *Chemistry* and *Atoms, Molecules, and Life* (AML) declare the same interest in their mutual subtitle, while the third, *Basic Chemistry of Life* (BCL), intends to provide a base in chemistry on which the student may build an understanding of the complex processes of life.

How are they to do this in less (BCL considerably less) than 1,000 pages? Clearly there must be selection and much of the treatment must be superficial. For example, atomic structure is treated by BCL in six pages and no student could have any basis for a further understanding of this subject after reading these. Both of the other books treat this topic in more detail, *Chemistry* making a valiant attempt to promote the ideas of quantum mechanics, whereas AML is rather cloudy on this issue.

Chemical bonding is initially introduced in all three texts by "dot formulae", *Chemistry* and AML developing the subject further to indicate the three-dimensional shape of molecules. All of the texts attempt to provide a foundation in physical chemistry, with an emphasis on those topics of interest to biologists. Electrolytes and non-electrolytes, solubility, osmosis and pH are all discussed in some detail and illustrated with biological examples; thus, for example, osmosis is described by the effect of changes in extracellular salt concentration on the red blood corpuscles. Each book includes a substantial section on organic chemistry, starting with methane and ending with nucleic acids and the genetic code. Both AML and BCL have extensive sections on biochemistry.

Of the three, BCL has the most medically orientated approach, the least basic chemistry and would require considerable elaboration on the part of the lecturer to give the student any real understanding of the subject. It virtually ignores the three-dimensional structure of molecules, and conformation is nowhere introduced. The consequences of three-dimensional structure is an area in which none of these texts is consistently satisfactory; thus although AML shows that the steroids are chair structures, it does not indicate how this influences the stereochemistry of the system, whilst *Chemistry*, though clearly indicating the stereochemistry, does not mention the conformational consequences. All of the texts use Haworth formulae for the carbohydrates and although *Chemistry* does introduce the conformational

formula for glucose it does not exploit it. *Chemistry*, as its title suggests, is most clearly directed towards the exposition of this science and, starting from the four elements and ending with global



biochemical problems, it represents an impressive attempt to encompass both the essential and the exciting new aspects of the subject within a single volume.

The print of all three books is excellent and chemical structures are clearly illustrated, *Chemistry* and AML in two-tone, BCL in monochrome. Each provides problems, often with answers, and *Chemistry* has available both a study guide for the student and a manual for the instructor. The latter provides answers for those questions without textual answers, test questions and suggestions for demonstrations. □

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Beyond description

H. Hassall

Basic Mathematics for Biochemists. By Athel Cornish-Bowden. Pp.137. Hbk ISBN 0-412-23000-3; pbk ISBN 0-412-23010-0. (Chapman & Hall/Methuen: 1981.) Hbk £7.95, \$17.95; pbk £3.95, \$8.95. *Quantitative Problems in Biochemistry*, 6th Edn. By Edwin A. Dawes. Pp.335. ISBN 0-582-44402-0. (Longman: 1980.) £13.95, \$32. *Biochemistry: A Problems Approach.* By William B. Wood *et al.* Pp.498. ISBN 0-8053-9840-6. (Benjamin/Cummings: 1980.) \$14.95, £8.95.

YEAR after year, with only one or two exceptions, the questions that are done least well in biochemistry examinations are those involving calculations and data assessment. Mass panic ripples through the examination rooms and those painstakingly constructed subtleties of the examiners, the diauxic inflections of the growth curve, the sigmoidicity of the Michaelis-Menten graph or the slight curvature of the Yphantis plot, are either missed or explained as experimental error.

If these three books have a common objective, as I believe they have, then it is to encourage a more quantitative treatment of the subject. However, each is different in its approach and has something unique to offer.

Basic Mathematics for Biochemists is the shortest of the three and has a straightforward aim — to rectify deficiencies in the mathematics needed to understand modern biochemistry. It begins reassuringly simply (almost too simply in that one is told that 2^2 means $2 \times 2 = 4$) but it does go on to cover integral and differential calculus, matrices and partial differentiation. The examples used have a biochemical origin and, taken as a whole, one is impressed by just how much information the author has been able to get into a book of 137 pages. Clearly, the students who assimilate its contents would have more than enough mathematics to cope with any first-degree course in biochemistry.

The book by Edwin Dawes, *Quantitative Problems in Biochemistry*, should need no introduction. It is now in its sixth edition and, coincidentally, in its sixth language. At the time of the first edition it must surely have been one of the first books of its kind, and the familiar style and content have altered only little over the years despite some changes in format. There is a new and useful chapter on membrane bioenergetics, this addition being balanced by a pruning of the text elsewhere. With respect to the problems themselves, the book relies heavily on the presumably well-tried and tested final examination questions from biochemistry degree courses in British universities. Few of these, however, have been updated since the last edition.

The third book, *Biochemistry: A Problems Approach*, has a different emphasis to the others. It is really a textbook of biochemistry using data assessment and questions to illustrate the principles, rather than just a book of problems with biochemistry as the source of material. The approach, while clearly not new, is refreshing. It has a chatty, personal style and although Fluhardy Pharmaceuticals, Dr F. Holding Munney and Dr Klassik appear (but only briefly) as the bodies faced with problems, the information that is conveyed is no less valuable; who knows, perhaps interest actually is more readily stimulated this way. Most of the questions are particularly searching and are balanced by full explanations in the answers. The text is comprehensive, ranging in 21 chapters from the "Clinical Characteristics of Living Matter" through to "Recombinant DNA and Genetic Engineering". The treatment is even, and while the book is not meant to replace standard texts such as Lehninger or Stryer, it complements them well and is good value at the price. □

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Physical chemistry: sugar in the medicine

S.P. Spragg

Physical Chemistry: With Applications to Biological Systems, 2nd Edn. By Raymond Chang. Pp.659. Hbk ISBN 0-02-321040-0; pbk ISBN 0-02-978355-0. (Collier Macmillan/Macmillan, New York: 1981.) Hbk £16.50, \$24.95; pbk £10.50. *Physical Chemistry for the Life Sciences*, 2nd Edn. By Garry M. Barrow. Pp.468. ISBN 0-07-003858-9. (McGraw-Hill: 1981.) \$32.95, £18.25.

IF ONE consults biological colleagues on what physical concepts they wish they had learnt as students, two extreme views appear: there are those who feel they prospered without needing physical chemistry, while others consider that physical chemistry is central to their branch of biology. The fact that two extremes are quoted by leaders in their fields confirms the view that some parts of biology can be pursued without an understanding of physical chemical principles, but if one is educating on a broad front then exposure to physical chemistry cannot be a bad thing. The question is, should we apply the old adage often used when preparing medicines, namely it must be good because it tastes so bad? Personally, I feel this is not necessary because all principles of physics and biology can be explained attractively and clearly despite their complexity. These thoughts were uppermost in my mind when reading the new editions of these two books.

Both texts are written in attractive styles and each depends on the working of simple numerical examples to illustrate the principles. The contents cover the full range of modern physical chemical ideas, carefully written using the properties of biological molecules for the examples. The authors have made considerable improvements over the first editions, mainly by expanding the discussions on thermodynamics. Both have rearranged the sequence of topics so that thermodynamics precedes spectroscopy. There are also more worked examples in the texts and more problems for the students to tackle (with answers at the end of the book); here Chang differs from Barrow since he has indicated which problems are more advanced. Chang has also expanded and moved the mathematical derivations of equations to the end of the appropriate chapters. Barrow follows a similar arrangement but unlike Chang he mixes mathematics with the particular applications of the chapter.

Both authors have introduced modern views into all topics, and I was particularly pleased to see that they have explained how transition state theory for solutions differs from that for a gas, as well as providing models for diffusion-controlled reaction (Chang is better here). They have also squeezed in descriptions of relaxation methods for studying rates of reactions.

As one would expect when comparing the products of two experienced teachers, there are differences in presentation: for example, Chang employs more diagrams than Barrow. My lasting impression was that Barrow contained more detail than Chang on subjects embraced by hydrodynamics; however, aspects such as wave-mechanics appeared to be more complex in Barrow than Chang. Also, I found that Chang's index was not as comprehensive as Barrow's. Yet these are details and students are as different as reviewers in their selection of material. So, since both books can be recommended, I suggest that prospective buyers dip into each of them before deciding which approach best suits their own personality. □

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Talking of organic chemistry

John Mann

The Vocabulary of Organic Chemistry. By Milton Orchin et al. Pp.609. ISBN 0-471-04491-1. (Wiley: 1980.) £18.75, \$43.75. *Introduction to Organic Chemistry*, 2nd Edn. By Andrew Streitwieser and Clayton H. Heathcock. Pp.1,258. Hbk ISBN 0-02-418050-5; pbk ISBN 0-02-978310-8. (Collier Macmillan/Macmillan, New York: 1981.) Hbk £18.95, \$28.95; pbk £9.95. *A Guidebook to Mechanism in Organic Chemistry*, 5th Edn. By P. Sykes. ISBN 0-582-44121-8. (Longman: 1981.) £7.50, \$16.95.

LINGUISTS have prepared lists of key words and expressions for most languages, but *The Vocabulary of Organic Chemistry* represents the first real attempt to do the same for organic chemistry. The intention of the authors was to "... identify the fundamental vocabulary of organic chemistry and then to present concise, accurate definitions with examples where appropriate ...", and in this they have been eminently successful. They define some 1,350 terms and concepts, with entries ranging in length from one line to a page.

The compendium is organized into 15 chapters, and commences with a concise introduction to symmetry, followed by a series of mathematical definitions pertaining to wave and quantum mechanics. In this second chapter the sections on bonding and orbitals are particularly good. Structural aspects of hydrocarbons are covered in Chapter 3, followed by a comprehensive list of organic

functional groups, including 35 containing sulphur and 24 containing phosphorus; this chapter thus provides a far better source of such information than any undergraduate textbook on the market.

Stereochemistry and conformational analysis appear in Chapter 5, and this compilation of terms is most timely since there is ever-increasing attention paid to the stereochemical aspects of organic chemistry in the research literature. In addition, many undergraduates have difficulty distinguishing between terms such as "stereoselective" and "stereospecific", or in understanding the significance of "prochiral hydrogens" and "asymmetric induction", and this chapter provides accurate and easily understood definitions for all of the commonly encountered terms.

Polyfunctional compounds are dealt with in Chapter 6, and this includes coverage of sugars and amino acid derivatives. Here, I feel too much stress is laid on Fischer projection formulae and there is scant mention of more realistic cyclic structures; also, the amino acid and protein section is too short to be really useful — surprisingly, the terms "enzyme" and "active-site" do not appear. Chapter 7 contains a useful collection of terms pertaining to separation techniques, following which there is excellent coverage of ions and electron deficient species. This is also timely since there is often confusion over the terms "carbocation", "carbonium ion" and "carbenium ion", and these are all unambiguously defined. Essential thermodynamics (Chapter 9) then precedes a classification of organic reaction mechanisms, and this latter chapter should be particularly useful for undergraduates since it also includes 15 pages encompassing topics such as orbital correlation diagrams, the Woodward-Hoffmann rules, and the frontier orbital approach to mechanism. It is interesting to note that the same number of pages is given over to these topics in Streitwieser and Heathcock, but the clarity of the coverage is greater in *The Vocabulary*.

The outstanding chapter then follows, in which 159 name and type reactions are discussed, with a mechanism given for each of them. This would make an excellent 90-page monograph in its own right; it certainly highlights the reason behind the oft-heard lament of the undergraduate: "There's too much to learn in organic chemistry". Chapter 12 is entitled "Organometal Chemistry", but contains little of relevance to organometallic chemistry. The term "cuprate", for example, does not appear, yet this is much more a part of the vocabulary of modern organic chemistry than are the terms "ligand field theory" and "edge-bridging ligand". Chapter 13 commences with a rather awkward definition of "natural product", and it would perhaps have been better to define the terms "primary metabolite" and "secondary metabolite".

This said, however, the chapter has good sections on cofactors and on all of the major biosynthetic pathways and classes of secondary metabolites. Finally, there are useful chapters on polymers and industrial processes, mainly those involving fossil fuels.

There is perhaps only one major omission in the book (and the authors recognize this), and that is coverage of spectroscopic methods. The book is beautifully produced with excellent structures throughout, and includes well-chosen examples and a good index. In parts it is reminiscent of a book of revision notes, but this may endear it to undergraduates. Overall, it is a unique and very useful reference work; all organic chemists should find something of interest within its pages.

Streitwieser and Heathcock have made a number of changes of organization for the second edition of their popular textbook, and the sections on organometallic chemistry, ^{13}C -NMR, and sulphur and phosphorus chemistry have been expanded. One omission is the useful appendix on functional group preparations

which was popular with undergraduates using the first edition. This book and Solomons's *Organic Chemistry* (for review see *Nature* **289**, 721) are two of the most popular organic texts on the market. There is little to choose between them, though the two-tone illustrations in Solomons perhaps give it a slight edge.

Finally, any book that has reached its fifth edition and been translated into six foreign languages must be rated a success. Such is the volume by Peter Sykes — a text that has probably been used by most chemistry undergraduates at one time or another. There were major revisions for the fourth edition, and the only important change for this edition is the inclusion of a chapter on linear free energy relationships, which further increases the utility of the book. It will be interesting to see if *The Vocabulary* is read as widely and proceeds through as many editions — I see no reason why it should not achieve this distinction. □

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Theory guides, but experiment decides

Gary O. Spessard

Spectral and Chemical Characterization of Organic Compounds: A Laboratory Handbook, 2nd Edn. By W.J. Criddle and G.P. Ellis. Pp.115. Hbk ISBN 0-471-27813-0; pbk ISBN 0-471-27812-2. (Wiley: 1980.) Hbk £13, \$30.90; pbk £4.50, \$10.70. *Operational Organic Chemistry: A Laboratory Course*. By John W. Lehman. Pp.671. ISBN 0-205-07146-5. (Allyn & Bacon: 1981.) \$24.95, £9.95.

THE task of introducing students to the experimental side of organic chemistry can be an awesomely difficult one, not only because of the wide variety of vocational goals and interests one encounters, but also because there are so many aspects of the subject which ought to be considered in any laboratory course. These aspects essentially centre on the synthesis and characterization of compounds. Criddle and Ellis in their *Spectral and Chemical Characterization of Organic Compounds* address the latter concern, while Lehman's *Operational Organic Chemistry* endeavours to cover both synthesis and characterization.

The second edition of Criddle and Ellis's text is only slightly modified from the first which appeared five years ago. For example, small additions and modifications appear in the notes explaining the chemical bases for the functional group tests in Chapter 2, in the section on interpretation of spectral data in Chapter 3 and in Chapter 5 where extensive tables of physical data are found. Using a step-wise approach, hints on how to interpret the results of chemical and spectral tests in

addition to compendia of physical data on compounds and their derivatives are presented in a compact style. While concise and informative, the book must be supplemented, as the authors point out, with more comprehensive works on the chemical bases of function group tests, the chemistry of derivatization and practical spectroscopy. No mention is made of the use of chromatography in analysing and separating mixtures, and only the briefest reference is given to safety in regard to handling reagents (often quite toxic) typical of the organic "qual" laboratory. As long as access to more complete sources of information is possible, this text can usefully serve as a guide for students involved in the qualitative analysis segment of a regular organic laboratory course or in one concerned with characterization alone.

Operational Organic Chemistry is another addition to the spate of laboratory manuals currently available. The author suggests that working in the laboratory involves performance of a series of operations such as filtration, extraction or spectral analysis from the start to the finish of an experiment. The student should gain an appreciation of these operations, described separately in the text, by learning to perform them within a series of preparative and analytical experiments. Superb background essays are included which encourage student involvement in the chemistry behind each experiment by suggesting roles (such as working as a chemist for a perfume manufacturer) students may assume while working in the laboratory. I

especially like the emphasis on pre-laboratory preparation whereby students are directed to read certain text sections and to complete specific exercises *before* working on an experiment.

The menu of practical exercises includes many of the "old reliables" as well as several new ones; suggestions for additional work are provided in the form of "minilabs" and "experimental variations". Potentially attractive to students with biologically-related interests is the extensive use of natural products because, as Lehman states, "they often cost less, smell nicer, and are more pleasant to work with than purely synthetic analogs". Safety is treated thoroughly and conscientiously throughout the book. I do have reservations, however, about the pedagogical benefits to be gained from the synthesis of azo dyes or the use of toluene-2,4-diisocyanate for polyurethane synthesis in light of the chemical hazards involved. The sections in the text on spectral techniques are adequate but not as good as those found in *Introduction to Organic Laboratory Techniques* by Pavia *et al.* Nonetheless, in spite of these minor criticisms, the book is rather unique in its approach and one which is well worth considering for adoption. □

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Atkins anew

Maurice Rigby

Physical Chemistry, 2nd Edn. By P.W. Atkins. Pp.1,095. Hbk ISBN 0-19-855150-9; pbk ISBN 0-19-866151-7. (Oxford University Press/W.H. Freeman: 1982.) Hbk £25, \$29.95; pbk £10.95. *Physical Chemistry*. By J. Philip Bromberg. Pp.882. Hbk ISBN 0-205-06572-4; pbk ISBN 0-205-06867-7. (Allyn & Bacon: 1980.) Hbk \$27.95, £22.25; pbk £9.95.

IN THE past few years several candidates have presented themselves as potential successors to established and widely-used general textbooks of physical chemistry such as W.J. Moore's *Physical Chemistry* (Longmans/Prentice-Hall, 1972) and G.M. Barrow's *Physical Chemistry* (McGraw-Hill, 1979). One of the most successful has been that of P.W. Atkins, first published in 1978 and rapidly followed by a corrected reprint, which now appears in a second edition. The first edition of this work was notable for a generally well-judged level of coverage of most aspects of the subject, and for the fresh and accessible presentation of difficult concepts.

In the new edition these desirable qualities have been retained, and some

omissions and slight imbalances have been rectified. There is a completely new chapter dealing with macromolecules — a subject sadly neglected in the first edition — and many figures have been changed, with the introduction of computer graphics. In addition the treatment of chemical bonding, which was previously rather brief, has been amplified, and the introductory material on statistical mechanics has been simplified. Comparisons between corresponding sections of the two editions reveal numerous small changes, supporting the claim that all sections of the book have been reconsidered. My overall impression is of an excellent textbook which has become even better, and which seems sure to enjoy continued popularity in the future. Who, in any case, could resist a physical chemistry book which considers (p.103) the musical implications of a dying ocarina player?

Against such competition, yet another general textbook will have to be very good if it is to wean readers away from the established sources. J. Philip Bromberg has taken up this challenge, but has not completely persuaded me. The author claims to have aimed to present the subject at the mid-college level, and has consciously refrained from producing an encyclopaedia of physical chemistry. For this ambition he should be congratulated — at least one recent text has seemed to be aimed more at the readership of *Annual Reviews of Physical Chemistry* than at an undergraduate audience.

The book follows a fairly well-worn path from the gas laws through thermodynamics, statistical mechanics, quantum mechanics and reaction kinetics, giving quite formal and detailed coverage of the necessary theoretical development though not neglecting to draw attention to the practical relevance of the subject, where appropriate. It is liberally interspersed with short biographical sketches of notable scientists, which may help to give students some feeling for the development of the subject. The overall balance appears to emphasize thermodynamics and quantum mechanics, which are respectively developed classically and axiomatically. Electrochemistry and surface chemistry appear as applications of thermodynamics, and there is no mention of catalysis or of non-equilibrium aspects of electrochemistry. The treatment of reaction kinetics is short and repeatedly uses a rather laborious analogy with molecular effusion, the merits of which are not immediately apparent. In short, although the book has some attractive qualities, and I shall try to arrange for a copy to be available in our college library, I do not believe that it will replace its rivals in a majority of chemistry departments. □

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NEW

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EARL L. GREEN

Director Emeritus of The Jackson Laboratory, Bar Harbor, Maine.
Editor, *Biology of the Laboratory Mouse*, Second Edition.

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ARTICLES

Convective growth rates of equatorial features in the jovian atmosphere

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The first results of measurements of divergence and vertical velocity of cloud features in the atmosphere of Jupiter are presented from analyses of Voyager images, performed using the interactive planetary imaging processing system at University College London. Active equatorial plumes are found to have typical divergences of $\sim(1-2) \times 10^{-5} \text{ s}^{-1}$, and vertical velocities of $20-40 \text{ cm s}^{-1}$, during the observational periods examined. These plumes are considerably more active than the bright albedo features in the turbulent regions of the jovian north and south equatorial belts.

OBSERVATIONS made by the Voyager imaging and IRIS instruments of the equatorial region of Jupiter have shown details of trains of white plume features moving in a westerly direction, with cloud material spreading out behind small bright centres of rising motion¹. The plume centres are positioned at about 9°N . A total of 13 features were seen at the time of the Voyager 1 encounter, with four active centres². Four months later, in July 1979, at the time of the Voyager 2 encounter, the structure of this region had changed so that only 11 features were evident and three had active centres³.

It has been suggested¹ that the plumes are mesoscale convective structures, formed as a travelling wave disturbance in a manner similar to that found in the Inter-Tropical Convergence Zone (ITCZ) of the Earth's atmosphere. Indeed, there are indications^{1,4} that these active plumes cause a major perturbation to the structure of the atmosphere from the cloud tops, throughout the troposphere and into the lower stratosphere.

This is a distance of at least 20 km, which is approximately the atmospheric scale height for Jupiter at this pressure level. A major disturbance to the atmosphere of this type must be associated with rapid vertical motion in the atmospheric levels beneath the visible clouds themselves. Furthermore, these plumes will have a significant effect on the energy budget of the equatorial region, through the mass transport of material associated with their morphological changes.

We estimate in this article, for the first time, the divergence characteristics of these plumes using Voyager images analysed on the University College London (UCL) interactive planetary image processing system (IPIPS)⁵. From this information, we calculate the vertical velocities which relate to the observed changes in the plume head structures. These new measurements provide valuable insight into the development and variations in these mesoscale structures, which have an important role in the equatorial meteorology of the jovian atmosphere.

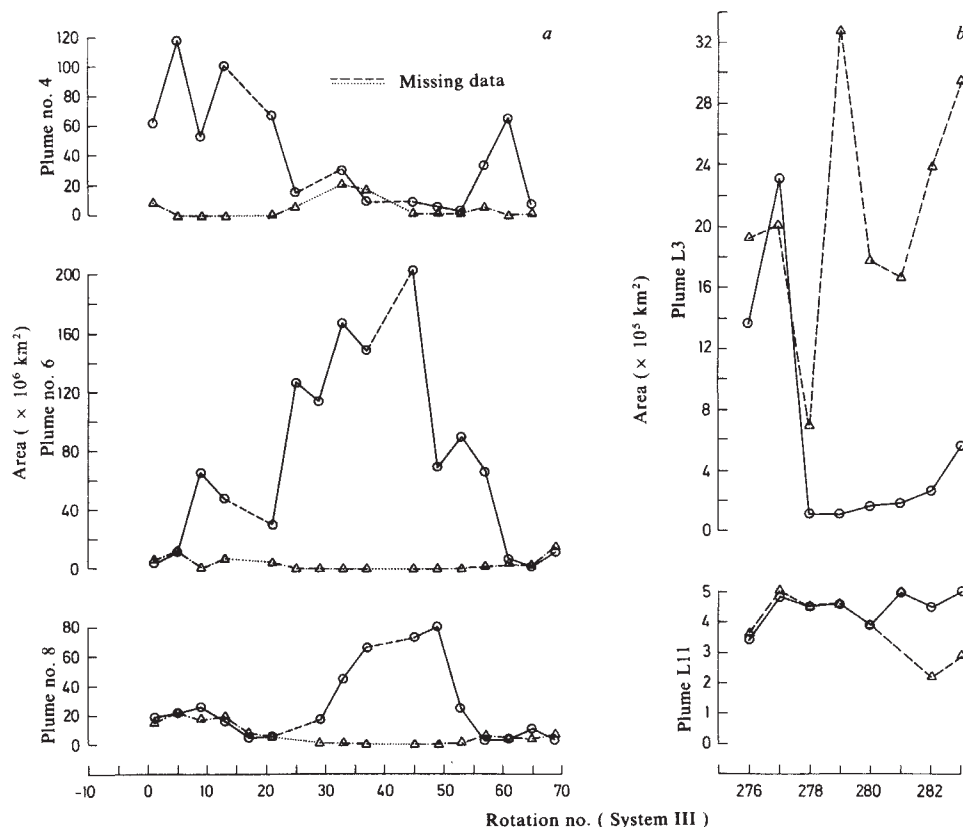


Fig. 1 The variation in the convective area of the three active plumes observed by Voyager 1(a) and for the two active plumes observed by Voyager 2(b) as a function of the rotation number for a single threshold level of brightness digital number (DN) = 185 (a, $\triangle$, $\triangle$, $\triangle$) (b, $\triangle$, $\triangle$, $\triangle$ DN = 195) and the 90% contour level ($\circ$) of instantaneous maximum brightness.

Observations and data analysis

Observations obtained by the Voyager imaging system every 72° long. (System III) during the observatory phases of each spacecraft encounter were used as a basis for this study. The measurements for this investigation were taken from blue filter Mercator projection mosaics made from rotation periods 1–70 (6 January–2 February 1979) for Voyager 1, when the spatial resolution was 438 km per pixel with a temporal resolution of four rotation periods. The rotation periods covered by Voyager 2 were 276–283 (24–28 May 1979), for which the spatial resolution was 138.46 km per line pixel, and the temporal resolution was every rotation period. The images used were violet filter cylindrical map projection mosaics. The rotation period reference system refers to the number of jovian days in System III longitude beginning with the first observations by the Voyager 1 spacecraft and continuing throughout the mission, concluding with the final measurements by Voyager 2. This is therefore a convenient scheme to represent the time variation of the observations during the 1979 Jupiter encounter.

To remove the effects of the spacecraft viewing geometry and the illumination conditions, the full disk images were first radiometrically decalibrated and then map projected for the Voyager 2 observations. The differences between these projections is negligible in the equatorial regions of Jupiter, where our analyses are performed. The Sun angle illumination effects were removed at the Jet Propulsion Laboratory from these images using a Lambert function for Voyager 1, and a Hapke function for the Voyager 2 images (see, for example, ref. 6). Then these photometrically decalibrated images were constructed into a mosaic to form sequential image maps of the entire planet for a full rotational period.

With the observations in this format, we can carry out the meteorological investigations using the analysis techniques developed and carried out at UCL. The bright albedo structures in the equatorial region certainly represent mesoscale con-

vective structure¹. In the terrestrial atmosphere, Sikdar *et al.*⁷ and Sikdar and Suomi⁸ have shown that the time variation of the visible brightness of a cloud anvil may be used to derive cloud-top divergences.

From the continuity equation, the rate of change of horizontal area, A , which represents the cross-section of the visible cloud system, is equal to the horizontal divergence. This may be written as:

$$\frac{1}{A} \frac{dA}{dt} = \text{div}_h V \quad (1)$$

Where $\text{div}_h V = \partial u / \partial x + \partial v / \partial y$, and u, v are the x, y components of velocity^{9,10}. Then the time and area averaged divergence, $\text{div}_h V$, is given by the expression

$$\frac{\ln(A_2/A_1)}{\Delta t} = \text{div}_h V \quad \text{s}^{-1} \quad (2)$$

where the area of the layer of outflow has increased from A_1 to A_2 during the time interval $\Delta t = t_2 - t_1$.

If we now use a scale analysis argument (see ref. 10) and assume that the vertical motion in the layer of outflow takes place over less than or equal to a scale height D , then the vertical velocity required to produce the observed divergence field is given by:

$$w \leq D \text{div}_h V = \frac{D \ln(A_2/A_1)}{\Delta t} \quad \text{ms}^{-1} \quad (3)$$

We consider the measurement of the time rate of change of area of the ammonia cirrus anvil above the region of the updraft to be representative of the layer of outflow for the convective storm system and subsequently referred to as the 'convective area'. Hunt *et al.*¹ suggest that the main driving mechanism for the equatorial plume systems is provided by the latent heat released in the layers of water clouds that are thought to exist up to three scale heights beneath the visible ammonia clouds. The depth of the total convective structure would therefore need to be a factor of two larger than terrestrial tropical cyclones.

Fraedrich *et al.*⁹ showed that for tropical cloud clusters, the area of the convectively active region of a cloud system may be calculated using two methods. Either (1) the brightness threshold remains constant throughout the sequence; or (2) the brightness threshold varies relative to the instantaneous maximum brightness in the cloud cluster. We have examined these two procedures for the Voyager plumes using the IPIS image processing facility⁵ and find that procedure (1) is unsuitable for these studies. Thus we have used procedure (2) to obtain divergence and vertical velocity estimates, as it was demonstrated to be more sensitive to the changes of the area of the convective core and not to gross brightness variations which were a function of the photometric decalibration, the mosaic image 'overlap' area of the errors inherent in the radiometric decalibration (see ref. 6).

Results

We have studied the three active convective structures visible in the equatorial zone in the Voyager 1 sequence. Figure 1a shows the considerable variation in the growth and dissipation of these active features. Over the 70 rotation periods observed, two of these features changed in area by a factor of 40. The two sets of curves refer to the calculated areas for a threshold of 185 (on a maximum grey scale of 255) and the 90% of maximum brightness contour level. The 90% contour level clearly shows the most active region of the convective structure, while the threshold reflects only gross brightness changes. There were apparently less active plumes observed during the time period studied in the second Voyager encounter¹. We have measured two active plumes, shown in Fig. 1b, and the 90% level seems again more representative of the areal changes in the convective area of these features than the threshold of 195. Once more, the rapid increase and decrease in the active areas is noticeable on relatively short time scales. From the resolution of the images

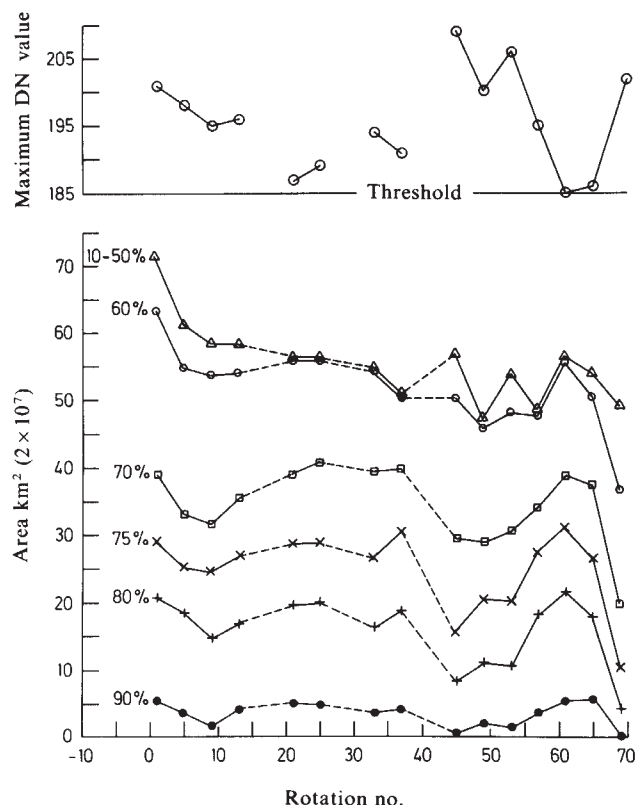


Fig. 2 The variation in the area of the bright albedo features in the turbulent region of the north equatorial belt (NEB) of Jupiter. Individual contours are shown separately for areas calculated at 10–90% of the maximum brightness level for each rotation period, together with the value of the instantaneous maximum DN level.

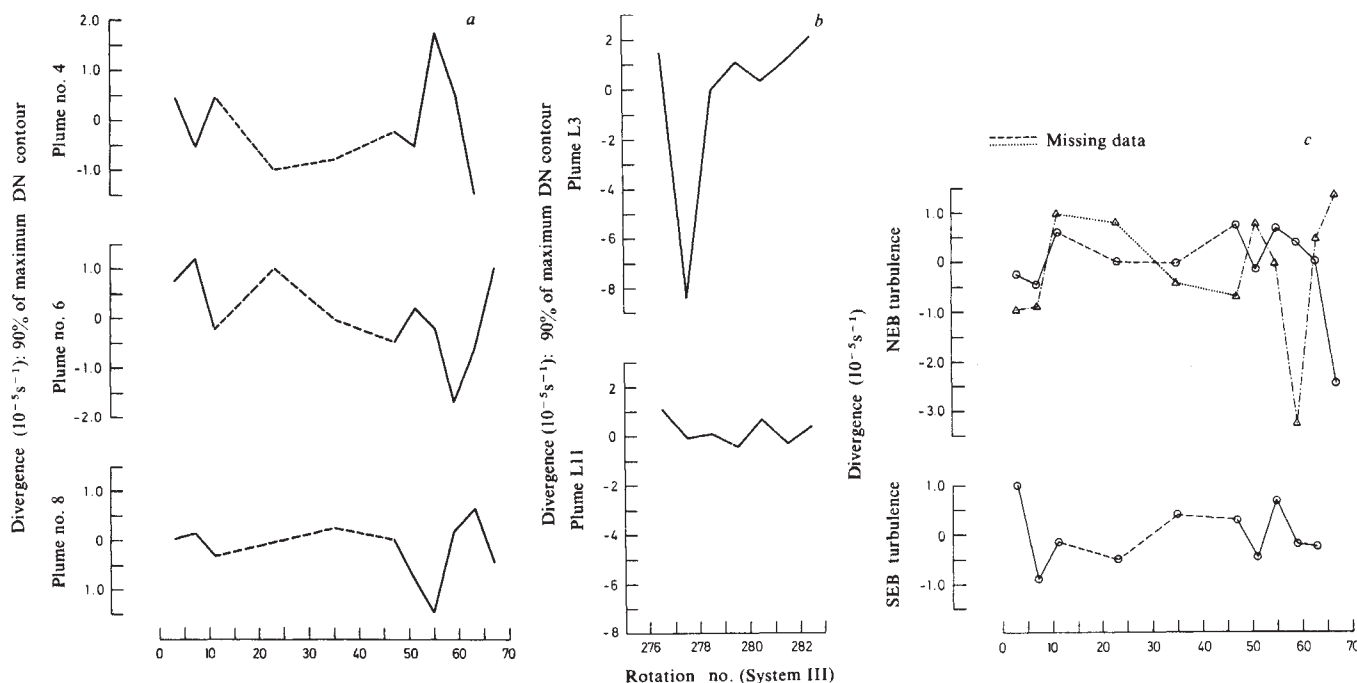


Fig. 3 The variation of divergence (10^{-5} s^{-1}) as a function of rotation number for: *a*, the three active plumes observed by Voyager 1; *b*, the two active plumes observed by Voyager 2; *c*, the bright albedo features in the turbulent regions of north and south equatorial belts. $\circ$, 90% maximum DN contour; $\triangle$, DN = 185 contour.

used in this study the smallest area that may be resolved corresponds to $\sim 4 \times 10^4 \text{ km}^2$. This is $\sim 10^{-2}\%$ of the area of a whole plume. Smaller scale structure, such as the core of the 'convective area' cannot be resolved with this data set.

There are also regions of rapidly changing small-scale bright albedo features in the north and south equatorial belts of Jupiter (see, for example, refs 2, 3). To determine whether the variations of these features have any similarity with the plumes, we have analysed them using the same procedures. Figure 2 illustrates the changes in area of these active regions of the north equatorial belt. We show the variation in area for various percentages of the brightness maximum of the region along with the maximum brightness and the threshold level of 185 used. Although the actual areas are similar in magnitude ($\sim 10^7 \text{ km}^2$ for the 90% level) to the plume feature, there is a smaller variation in the area of the north equatorial belt. A similar situation is apparent from our analysis of the features in the south equatorial belt.

These changes in the convective areas of the plumes and features in the north and south equatorial belts may then be used to calculate the divergence from equation (2). Figure 3*a* shows the divergence variation for the three active plumes observed by Voyager 1. The maximum divergences are not in phase with one another and their values are not identical: the maximum value for plume 4 is $1.75 \times 10^{-5} \text{ s}^{-1}$ at rotation 55, while for plumes 6 and 8 the values are $1.25 \times 10^{-5} \text{ s}^{-1}$ at rotation 67, and $0.7 \times 10^{-5} \text{ s}^{-1}$ at rotation 63, respectively. The features observed during the Voyager 2 encounter have divergences of about $(1-2) \times 10^{-5} \text{ s}^{-1}$. On rotation 1, the 'active' centres of plumes 4, 6 and 8 were located at approximately 138° , 197° and 267° long. (System III). On rotation 276 the 'active' centres of plumes L3 and L11 were located at 317° and 131° long. (System III).

The negative values of divergence which appear in Fig. 3*a*, do not correspond to convergences, but to evaporation at the edges of the plume heads.

The analyses by Hunt *et al.*¹ and Conrath *et al.*⁴ have shown that the plumes are mesoscale disturbances that cause a significant perturbation to the vertical temperature structure throughout the jovian troposphere above the visible clouds and into the lower stratosphere. It is possible that the entire region from 50 to 1,000 mbar may contain clouds of ammonia conden-

sates of varying opacities as Sato and Hansen¹¹ suggest. The clouds which constitute the plumes, would therefore be contained in a larger portion of this region and may extend more than 40 km from the base of the water cloud to the base levels of the ammonia cirrus anvil. The anvil cloud itself will extend at least a further 20 km which is consistent with jovian cloud models¹². The outflow from the convective disturbance which we see in the Voyager images as the plume tail is more than 500 times the possible vertical depth of the plumes. The jovian plume then closely resembles the terrestrial large scale convective cloud system, but on a much greater scale.

We therefore scale the measured divergence values by the minimum depth of the convective flow, which we estimate to be about 20 km. With this value, we see that the larger vertical velocities are about $20-40 \text{ cm}^{-1}$. These values represent the vertical motion of an area of the plumes whose cross-section is several thousand kilometres and certainly consistent with the values found in terrestrial convective storms (see for example, ref. 10). Note that Adler and Fenn (see ref. 12) showed that the maximum updraft may be as much as two orders of magnitude greater than that implied by the scaling argument used here. Our derived values may therefore be an underestimate of the true vertical motions. Furthermore, the magnitude of vertical motions, associated with individual cloud turrets in the convective area, would certainly be large enough to create 'horizontal' gravity waves downstream of the convective activity, as was observed¹³ in the tail of one of these active plumes.

The major driving mechanism for these features must come from the latent heat released by condensation of water in the cloud layer, located some three density scale heights beneath the visible convective area¹². The modelled top of the ice cloud was shown to be at a temperature of around 210 K (ref. 12). Hunten *et al.*¹⁴ have modelled the observed IR opacities and produced a vertical temperature profile which shows that at this temperature the pressure is at ~ 2.5 bar. In addition, their calculations show that the dominant heating up to the 2 bar level is solar. At higher pressures, internal heating is the only possible source for triggering convective activity. It has been suggested¹⁵ that only small amounts of internal heating anomalies are required to produce vertically propagating gravity waves which may trigger

convective activity. This may lead to further sub-cloud convergence due to the convective environment initiating in a wave-mean flow interaction. This could then provide the necessary criteria for setting up an equatorially travelling-wave disturbance.

Conclusions

We have measured for the first time the growth rate of convective cloud features in the equatorial region of the jovian atmosphere. The equatorial plumes seem to possess many of the characteristics of the large-scale convective cloud systems observed in the Earth's tropical regions. The vertical velocities associated with these features are similar in magnitude to the terrestrial systems. However, the jovian clouds occupy a much larger spatial scale.

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The disturbances seen in the turbulent regions of the north and south equatorial belts are much less active than the equatorial features. It is possible therefore that these features are shallower in this region of the atmosphere. Furthermore, this situation may also be related to their shorter lifetime.

In both situations, the convective activity seems to be generated from travelling wave disturbances in the jovian atmosphere. The intensity of the convection in the equatorial plumes and the magnitude of the divergence and vertical velocities derived for these features is consistent with them being generated by large-scale convergence in deep cloud layers beneath the visible surface of the planet.

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Glacial to interglacial contrasts in the northern Indian Ocean

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Differences between the $^{18}\text{O}/^{16}\text{O}$ ratios of planktonic foraminifera deposited during the last glacial maximum, about 18,000 yr ago, and those deposited during the Holocene show that in the Indian Ocean, the south-west monsoon was weaker than today but that the north-east monsoon was stronger. The upwelling observed in modern conditions along the southern coast of Arabia had disappeared because of the low speed of the southwestern winds during glacial summers. The reduction in rainfall and runoff over the continent caused a reduction of the salinity gradient in the Bay of Bengal and along the western coast of India. Increased precipitation fell on the sea south of 10°N , while strong evaporation over the northernmost Arabian Sea produced an enhanced salinity gradient in that area.

THE seasonally varying monsoon circulations associated with the annual heating and cooling of the Asian continent are important aspects of the general circulation of the atmosphere. During the summer, as solar radiation warms the land and the ocean, the overlying air is heated by conduction and expands because the land heats faster than the ocean. The warmer air over the land rises and is replaced by denser ocean air which carries moisture evaporated from the ocean. When the moist air moves inland, it too rises and its water vapour condenses causing heavy rain over the continent. The circulation of the monsoon winds is deflected by the rotation of the Earth through the Coriolis force, which deflects winds to the right in the Northern Hemisphere so that summer monsoon winds essentially blow from the south-west. With the onset of winter, both the land and the ocean lose heat by radiation to space. The greater heat capacity of the ocean results in a greater cooling over the continent than over the ocean so that dry winds blow from the continent to the ocean. Due to the rotation of the Earth, winter winds blow from the north-east. The north-east monsoon continues in a steady state until solar heating of the spring dissipates the temperature gradient that powers the winter monsoon.

This monsoon climate that prevails over the northern Indian Ocean produces large salinity gradients in surface waters, although sea-surface temperatures, which are largely determined by the amount of radiation received from the Sun, are much less variable. As freshwater is depleted in the heavy

isotopes of oxygen compared with seawater, the modern salinity pattern for surface waters is closely matched by the oxygen isotopic composition of recent Holocene calcitic tests of surface dwelling foraminifera such as *Globigerinoides ruber*. During the

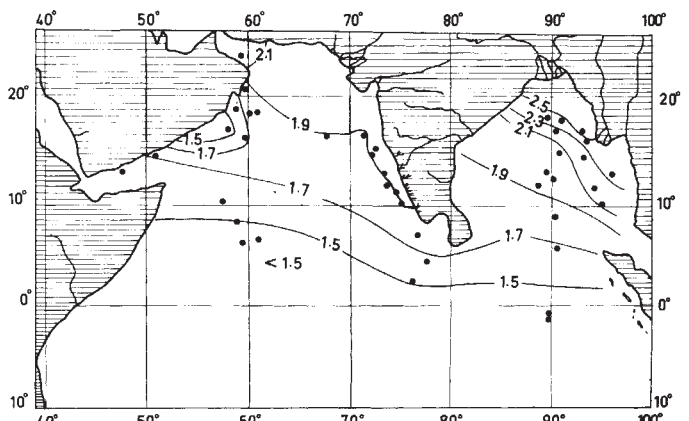


Fig. 1 a, Map of mean annual sea-surface salinity in modern times computed from monthly data collected by Wyrtki³. b, Map of *G. ruber* $\delta^{18}\text{O}$ values during Holocene time. ●, Location of deep-sea core samples. *, Location of trigger weight core-top samples. c, Map of *G. ruber* $\delta^{18}\text{O}$ values at the last glacial maximum. iso- $\delta^{18}\text{O}$ lines have been drawn by hand by interpolating between the control points.

last ice age, which reached its maximum ~18,000 yr ago^{1,2} only small changes in the northern Indian Ocean temperature pattern have been recorded²⁻⁴, but important variations in the precipitation pattern over the surrounding continents have been evident⁵⁻⁷. I now reconstruct the major hydrological trends, essentially that of salinity, in the northern Indian Ocean during the last glacial maximum by comparing maps of the oxygen isotopic composition ($\delta^{18}\text{O}$) of planktonic foraminifera that lived in surficial waters during the Holocene and at the last glacial maximum.

Modern hydrological setting

Today, the northern Indian Ocean is characterized by a strong salinity contrast between its two major basins, the Arabian Sea and the Bay of Bengal⁸. The heavy summer monsoon rain above the south-west Asian continent is collected by major rivers (Ganges, Brahmaputra, Irrawaddy and Salween) which drain into the northern end of the Bay of Bengal and the Andaman Sea. Because this area receives a large excess of direct precipitation, its low salinity surface water contrasts sharply with high salinity surface water of the Arabian Sea where evaporation is high (Fig. 1a). Due to this general pattern of evaporation-precipitation, isohalines show generally east-west trends, but along the western coast of India they turn north-south. This local pattern is due to two major freshwater inflows⁹. The first is the heavy rain which falls above the western flank of the Dekkan Mountains during the summer monsoon and is drained to the sea along the coast of Kerala, south-west India, by a dense net of mountain rivers. The second is the transport of low-salinity water from the Bay of Bengal into the eastern Arabian Sea during the north-east monsoon. Thus low-salinity water is found along the coast from July to February¹⁰, and means that the general pattern of mean annual sea-surface salinity in the northern Indian Ocean is closely related to the monsoon circulations.

The sea-surface temperature gradient is much weaker than that of salinity. During the north-east monsoon, a rather gentle phenomenon, the oceanic circulation is only moderately strong⁸. No striking upwelling areas develop and the sea-surface temperature pattern is essentially zonal, with temperature decreasing progressively towards the north¹⁰. The more vigorous atmospheric and oceanic circulation during the south-west monsoon causes intense upwelling along the coast of Arabia. This upwelling which brings cool, nutrient-rich deep water in the surface, is characterized by a strong drop of sea-surface temperature which falls below 24 °C in the proximity of the coast of Arabia north of 15° N (ref. 11). This produces a strong temperature gradient perpendicular to that coast. As the upwelling is restricted to the summer period, when the winds blow parallel to the coast, the sea-surface temperature gradient linked with the coastal upwelling is another good indicator of the intensity of the south-west monsoon circulation.

Isotopic strategy

The modern hydrological setting of the northern Indian Ocean is closely related to the monsoon. Therefore, the reconstruction of historical geographical variations of sea-surface salinity and upwelling areas should be an effective way of estimating the intensity of the south-west monsoon in climatic conditions different from those of today. Present palaeoceanographic reconstructions of the Arabian Sea and the Bay of Bengal essentially deal with sea-surface temperature²⁻⁴, which show upwelling variations during the last climatic cycle^{2,3,12}, but past sea-surface salinity has been estimated along only one north-south transect in the Bay of Bengal¹³. We have mapped the geographical distribution of the oxygen isotopic composition ($\delta^{18}\text{O}$) of planktonic foraminifera that lived in surficial waters during Holocene time and at the last glacial maximum. Planktonic foraminiferal $\delta^{18}\text{O}$ is controlled in a complex way by sea-surface water $\delta^{18}\text{O}$ and temperature¹⁴⁻¹⁷. In upwelling areas, the $\delta^{18}\text{O}$ variations should mainly reflect the sea surface temperature gradient, the slope of which is linked to the

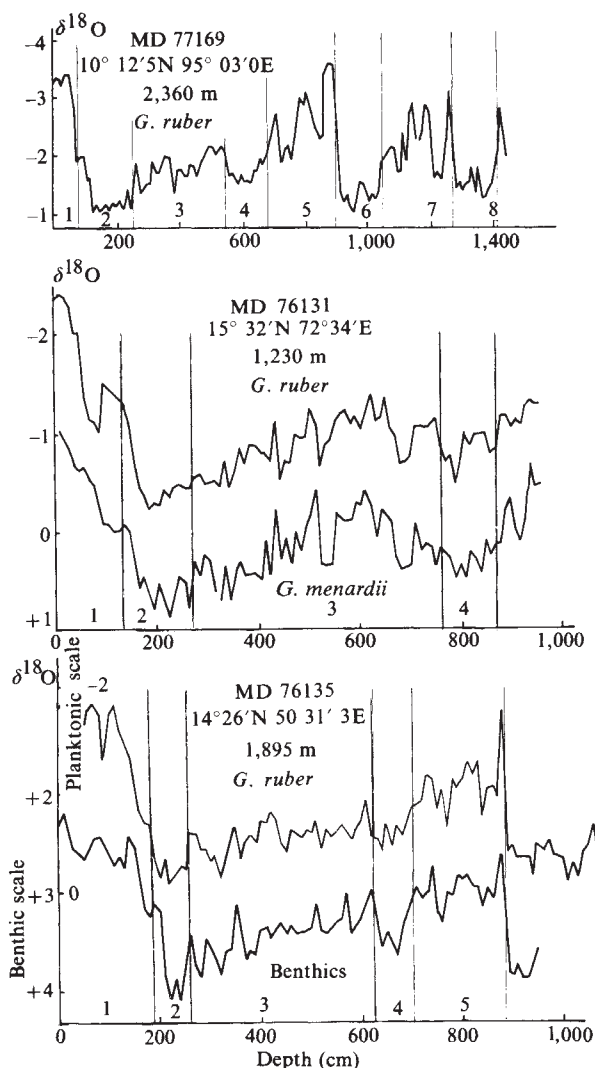


Fig. 2 Isotopic record of three cores from the northern Indian Ocean: cores MD 77169 (the Andaman Sea), MD 76131 (the Eastern Arabian Sea) and MD 76135 (the Gulf of Aden). Odd and even numbers refer to interglacial and glacial stages as defined by Emiliani²⁷. *Globorotalia menardii* is a deep foraminifera which, like benthic foraminifera, is not sensitive to the variations of temperature and salinity in the euphotic zone¹⁴.

intensity of upwelling and the wind strength. Except for the Arabian coast, upwelling is not significant in the northern Indian Ocean, and temperature gradients are small in modern conditions¹⁰, and have remained small during the Upper Quaternary while the global climate varied^{1,2,4}. Hence geographical variations of planktonic foraminifera $\delta^{18}\text{O}$ in the northern Indian Ocean should essentially reflect the sea-surface water $\delta^{18}\text{O}$.

Surface waters of the oceans have widely different $\delta^{18}\text{O}$ which are related to salinity¹⁷⁻²⁰. In general, tropical waters have a high salinity and are isotopically heavy, owing to the preferential loss of the isotopically light molecule H_2^{16}O and the concentration of the isotopically heavy molecule H_2^{18}O in the residual sea-surface water. In the atmosphere, cooling of the vapour causes condensation and precipitation. The oxygen isotopic composition of the first condensed rain will be enriched in ^{18}O compared with the atmospheric water vapour, but its $\delta^{18}\text{O}$ will be slightly lower than that of surficial sea water²¹ due to the low condensation temperature and the relative humidity of the atmosphere which is far from saturation¹⁸. A further ^{18}O depletion in rain falling over the continent can be explained by considering the formation of precipitation as a Rayleigh process, whereby with continuing precipitation from an air mass the remaining water vapour is progressively depleted in ^{18}O (refs 18, 21).

Hence a linear relationship between sea-surface salinity and $\delta^{18}\text{O}$ is observed locally. The gradient of the isotope-salinity relationship depends on the mode of formation of the freshwater arriving into the sea: it is small for a first condensation rain falling over the ocean, whereas it is much larger for the outflow of rivers fed by rain falling over cold mountains^{18,21,22}. This slope can be determined experimentally in modern conditions, but we have no way to determine it accurately in the past. Hence I consider only a qualitative discussion.

Data base

The oxygen isotopic composition of the foraminiferal species *G. ruber* was measured in 41 piston cores and 36 core-top samples from the Arabian Sea, the Bay of Bengal and the Andaman Sea, of which 12 are from ref. 3. This species was chosen because it secretes most of its carbonate in the surficial mixed layer¹⁷. About 30–40 shells were separated for each stable isotope analysis. The shells were roasted under vacuum at 50 °C and evolved CO_2 was analysed in a VG Micromass mass spectrometer^{23,24}.

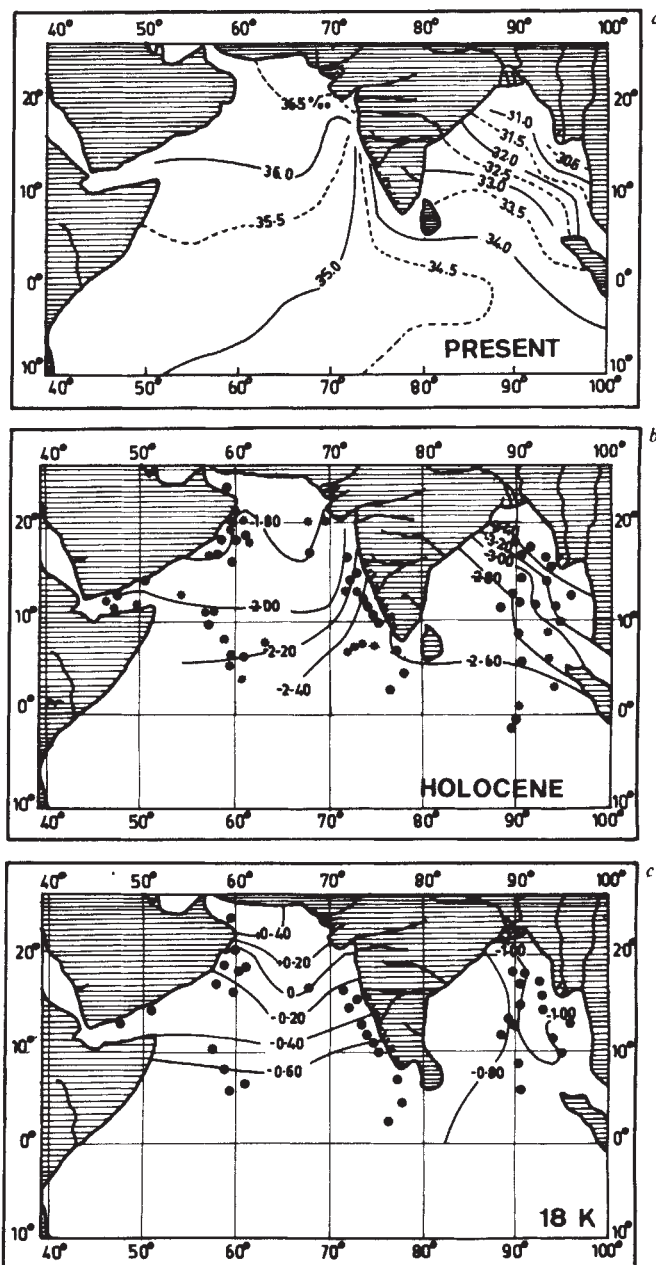
Downcore analyses were performed every 10 cm to obtain an accurate stratigraphy for the deep-sea sediments. Isotopic stratigraphy is based on the demonstration that downcore variations in the oxygen isotope composition of foraminiferal shells reflect primarily variations in the continental ice volume^{23,25,26}.

Table 1 Location of piston cores used for the Holocene and last glacial maximum reconstructions

Core	Lat.	Long. E	Depth (m)	$\delta^{18}\text{O}$ Holocene	$\delta^{18}\text{O}$ Glacial
MD 76127	12°05' N	75°54'	1,610	-2.17	-0.54
MD 76128	13°08' N	73°19'	1,712	-2.44	-0.46
MD 76129	15°00' N	72°20'	1,954	-2.28	-0.35
MD 76131	15°32' N	72°34'	1,230	-2.38	-0.26
MD 76132	17°00' N	71°31'	1,430	-2.13	-0.24
MD 76135	14°27' N	50°31'	1,895	-1.90	-0.18
MD 76136	12°52' N	46°49'	1,649	-1.81	-0.28
MD 77169	10°13' N	95°03'	2,360	-3.14	-1.02
MD 77171	11°46' N	94°09'	1,760	-3.24	-0.99
MD 77176	14°31' N	93°08'	1,375	-3.18	-1.17
MD 77177	16°25' N	93°24'	2,284	-3.41	-1.02
MD 77178	17°12' N	93°05'	2,459	-3.70	-1.12
MD 77179	18°22' N	91°01'	1,986	-3.50	-1.02
MD 77180	18°28' N	89°51'	1,986	-3.41	-0.90
MD 77181	17°24' N	90°29'	2,271	-3.34	-0.99
MD 77191	07°30' N	76°43'	1,254	-2.61	-0.84
MD 77194	10°28' N	75°14'	1,222	-2.56	-0.68
MD 77195	11°30' N	74°32'	1,426	-2.55	-0.57
MD 77200	16°33' N	67°54'	2,910	-1.77	+0.13
MD 77202	19°13' N	60°41'	2,427	-1.92	-0.08
MD 77203	20°42' N	59°34'	2,442	-1.79	+0.07
MD 77204	19°18' N	58°26'	1,430	-1.71	-0.13
ORKS 08	23°28' N	59°11'	2,700	-1.58	+0.55
ORKL 10	17°29' N	37°30'	2,390	-1.71	-0.30
RC 9 162	19°05' N	60°25'	3,092	-1.94	-0.17
RC 12 339	09°08' N	90°02'	3,010	-2.65	-0.93
RC 12 340	12°42' N	90°01'	3,012	-2.84	-1.04
RC 12 341	13°03' N	89°35'	2,988	-2.71	-0.75
RC 12 343	15°10' N	90°34'	2,666	-2.84	-1.04
RC 12 344	12°46' N	96°04'	2,140	-3.28	-0.88
RC 14 35	00°50' S	89°57'	3,021	-2.25	-0.51
RC 14 36	00°28' S	90°00'	3,076	-2.25	-0.93
RC 14 39	05°51' N	90°31'	2,952	-2.64	-0.94
V 14 101	08°39' N	58°34'	2,849	-2.10	-0.54
V 14 102	10°15' N	57°11'	3,915	—	-0.67
V 19 185	06°42' N	59°20'	2,867	-2.16	-0.70†
V 19 188	06°52' N	60°40'	3,356	-1.82	-0.55
V 29 15	11°57' N	88°44'	3,173	-2.68	-0.74
V 29 29	05°07' N	77°35'	2,673	-2.36	-0.68
V 29 30	03°05' N	76°15'	3,651	-2.34	-0.81
V 34 88	16°31' N	59°32'	2,120	-1.94	-0.27

The oxygen isotopic composition of *G. ruber* for Holocene and glacial times are reported as $\delta^{18}\text{O}$ versus PDB.

† Value taken from ref. 2.



the sediment cores can be recognized from the planktonic record as well as from the benthic record.

The synchrony of the Indian Ocean isotopic records was recently checked by ^{14}C dating in six cores². The mean age of the maximum glaciation was $\sim 18,000$ yr BP with a standard deviation of 1,500 yr. If the climate and the global ice volume were changing rapidly around 18,000 yr BP, there might be serious inaccuracies in the estimates of the $\delta^{18}\text{O}$ value at the last glacial maximum. However, high sedimentation rate deep sea cores suggest that the interval 22,000–15,000 yr BP was characterized by relatively stable conditions^{24,27–29}.

Holocene reconstruction

The data used to construct the Holocene map (Fig. 1b) represent the mean value of Holocene levels for each piston core: digital values are reported in Table 1. In four cores, the uppermost levels show evidence of strong dissolution and a correlative $\delta^{18}\text{O}$ increase during the end of the Holocene. Such an increase can be explained by the preferential removal through dissolution of fragile individuals^{31,32} whose tests were not overlain by a thick calcitic crust deposited during gametogenesis. As gametogenetic calcification occurs at great depth, below the euphotic zone, and in cold water, the tests under the thicker crust are also isotopically heavier³³. Hence the fossil population remaining in a deeply dissolved sediment is isotopically heavier than the undissolved fauna. Such a $\delta^{18}\text{O}$ increase is not evidenced in other nearby cores that are free from dissolution. Therefore the enriched levels were rejected in calculating the mean Holocene $\delta^{18}\text{O}$ value. Core top samples were also used to complete the Holocene map but stratigraphical control for those samples is not available and some scatter is inevitable in such a data set. Nine core-tops were rejected because their $\delta^{18}\text{O}$ did not fall within the range of values for nearby core that were verified by oxygen isotope stratigraphy. These samples were collected from the 90° E ridge and from the central Arabian Sea, where sedimentation rates are very low, and probably did not represent the most recent sediment. Consequently, iso- $\delta^{18}\text{O}$ map for the Holocene (Fig. 1b) is based on measurements of *G. ruber* $\delta^{18}\text{O}$ from 68 locations. The standard deviation of a single analysis is 0.07‰ (ref. 24). Although most $\delta^{18}\text{O}$ values were calculated as mean value of several intervals, variations of 0.1‰ were not considered significant and iso- $\delta^{18}\text{O}$ lines have been drawn separated by 0.2‰.

The map of *G. ruber* $\delta^{18}\text{O}$ in the northern Indian Ocean during the Holocene (Fig. 1b) reveals the major features of the mean annual salinity in modern conditions. In the Bay of Bengal, the $\delta^{18}\text{O}$ values decrease progressively towards the mouth of the Ganges, Brahmaputra, Irrawaddy, Salween River system, clearly reflecting the salinity pattern. The low-salinity waters along the southwestern coast of India are also well recorded in the *G. ruber* $\delta^{18}\text{O}$ values that are $\sim 0.5\%$ isotopically lighter than values from the same latitude in the central and western Arabian Sea. The general north–south $\delta^{18}\text{O}$ increase in the Arabian Sea also fits the salinity pattern and reflects the strong evaporation excess in this basin. The only misfit between the salinity and $\delta^{18}\text{O}$ patterns is observed in the western Arabian Sea along the coast of Arabia. As the oxygen isotopic composition of the foraminifera shells depends on the seawater temperature, the observed *G. ruber* $\delta^{18}\text{O}$ increase reflects the strong temperature drop due to coastal upwelling which brings cool, nutrient-rich deep waters in the surface³⁸. To summarize the Holocene pattern, from south to north, *G. ruber* $\delta^{18}\text{O}$ values decrease by 1.1‰ in the Bay of Bengal and increase by 0.8‰ in the Arabian Sea whereas mean annual sea-surface salinity decreases by 3‰ in the Bay of Bengal and increases by 1.5‰ in the Arabian Sea. The proportionality coefficient between $\delta^{18}\text{O}$ and salinity is not the same in the two basins because the mechanisms which produce the isotopic changes are different: dilution with freshwater in the Bay of Bengal and evaporation in the Arabian Sea.

Isotopic pattern during the last glacial maximum

$\delta^{18}\text{O}$ values for the last glacial maximum (Fig. 1c) were calculated as mean values of the isotopic stage 2 maximum and are reported in Tables 1 and 2. Dissolution was systematically lower during glacial time than during Holocene time and no sample was rejected for dissolution bias, so that iso- $\delta^{18}\text{O}$ map for glacial conditions is based on measurements of *G. ruber* $\delta^{18}\text{O}$ for 41 locations.

Glacial *G. ruber* $\delta^{18}\text{O}$ values are significantly heavier than those of Holocene time. This strong isotope enrichment primarily reflects the waxing of large continental ice-sheets, depleted in ^{18}O , and which extensively covered northern Europe and North America^{1,25,26}. The amplitude of this glacial effect is $\sim 1.6\%$ (refs 24, 28, 34), the remaining part of the ^{18}O enrichment is due to local surface seawater salinity and temperature changes. Temperature changes have been rather small in the northern Indian Ocean during the past 25,000 yr (ref. 2). At the last glacial maximum, mean annual sea-surface temperatures for the Bay of Bengal were $\sim 1^\circ\text{C}$ lower than modern values, while those from the Arabian Sea were almost the same or warmer than those of today. Geographical variations of *G. ruber* $\delta^{18}\text{O}$ values at the last glacial maximum will therefore mainly reflect the mean annual sea-surface salinity pattern.

The ice-age *G. ruber* $\delta^{18}\text{O}$ map (Fig. 1c) reveals striking differences with the modern pattern.

(1) A marked reduction in the $\delta^{18}\text{O}$ gradient observed in the Bay of Bengal and the Andaman Sea is probably associated with

Table 2 Location of core-tops and oxygen isotopic composition of *G. ruber* used for the Holocene reconstruction

Core	Lat. N	Long. E	Depth (m)	$\delta^{18}\text{O}$ Holocene
A 15585	20°09'	69°26'	216	–1.85*
A 15586	20°07'	67°55'	3,047	–1.87*
A 15591	21°00'	59°33'	1,267	–1.48*
A 15592	20°50'	61°01'	2,628	–1.55*
A 15596	18°56'	61°23'	3,694	–1.80*
A 15597	17°26'	57°11'	1,805	–1.50*
A 15612	13°35'	71°34'	1,697	–1.95*
Dodo 204	03°09'	94°06'	2,150	–2.57
MD 77164	06°05'7	93°37'3	2,140	–2.89
MD 77185	12°24'	92°03'7	1,461	–3.07
MD 77197	13°11'	73°26'	1,260	–2.46
RC 9 155	07°24'	72°48'	1,765	–2.57
RC 9 161	19°34'	59°36'	3,332	–1.61*
RC 12328	03°57'	60°36'	3,087	–2.33
RC 12347	09°20'	93°20'	1,533	–2.78
RC 14 37	01°28'	90°10'	2,226	–2.86
RC 17126	07°—	72°—	1,723	–2.47
V 14103	11°27'	56°14'	4,232	–2.00
V 14104	13°26'	53°27'	2,670	–1.98
V 14106	12°52'	48°25'	2,144	–2.17
V 14107	11°51'	46°45'	1,608	–2.23
V 14108	12°37'	45°38'	1,176	–2.41
V 19177	07°35'	74°13'	2,770	–2.37
V 19178	08°07'	73°15'	2,188	–2.49/–2.58*
V 19183	08°07'	62°47'	4,451	–2.09
V 34 80	06°07'	59°26'	3,292	–1.85
V 34 85	11°48'	57°37'	3,190	–1.86
A 15558	08°59'	51°44'	3,985	–1.44†
Dodo 197	02°58'	88°51'	4,017	–1.73†
Dodo 200	02°55'	91°03'	3,472	–2.15†
Dodo 201	02°59'	91°41'	4,131	–2.26†
RC 12329	02°59'	65°12'	3,864	–1.09†
RC 12331	02°30'	69°52'	3,941	–1.44†
RC 14 39	05°51'	90°31'	2,952	–2.42†
V 19176	07°07'	76°33'	1,773	–2.34†
V 19184	07°26'	61°04'	3,471	–0.58†

* Value taken from ref. 3.

† Rejected value.

a decrease in river discharge into the northern end of this basin. The isotopic result supports independent sea-surface salinity estimates deduced from faunal analysis¹³. The shape of the tongue of low salinity water (marked by the line $\delta^{18}\text{O} = -1\%$) suggests that in the Bay of Bengal the clockwise circulation linked to the north-east monsoon³⁵ dominates the mean annual water circulation. The occurrence of a strong north-east monsoon during glacial conditions is supported by independent pollen evidence³⁰.

(2) The glacial *G. ruber* $\delta^{18}\text{O}$ pattern for the Arabian Sea is much more zonal than the modern one because within the same latitudinal belt, *G. ruber* $\delta^{18}\text{O}$ values are similar. This uniform latitudinal distribution is related to the disappearance of both the low salinity-low $\delta^{18}\text{O}$ area along the southwestern coast of India and the cool-high $\delta^{18}\text{O}$ upwelling area along the coast of Arabia³. Both trends are likely to be associated with a reduction of the south-west monsoon wind regime.

(3) A marked increase in the *G. ruber* $\delta^{18}\text{O}$ gradient is observed in the Arabian Sea. This strong glacial isotope gradient which is larger than 1‰ (compare with the modern one which is only 0.8‰) is not related to the geographical distribution of the sea-surface temperatures which have been shown to be more uniform than today²⁴ but indicates a strong salinity increase from south to north. The map of the amplitude of $\delta^{18}\text{O}$ change between the last glacial maximum and the Holocene (Fig. 3) shows marked latitudinal variations. In the Arabian Sea, this amplitude is lower than the ice volume effect alone (1.6‰) south of 10° N. Such a low amplitude is not due to bioturbation as it is found in cores with sedimentation rates larger than 2–3 cm kyr⁻¹ (core V 19 188; V 2930). Because no major rivers outflow south of 10° N in the northern Indian Ocean, this low amplitude would reflect a marked precipitation increase over oceanic regions south of 10° N. Towards the north, the glacial-interglacial $\delta^{18}\text{O}$ amplitude increases. In the Bay of Bengal, this pattern clearly reflects the low river discharge during glacial conditions. In the Arabian Sea, it reflects stronger evaporation over its northernmost area and the Gulf of Oman. This stronger evaporation is probably linked to reinforced dry northeastern winds which blew from the continent to the ocean and to the warming of the Arabian Sea resulting from the reduction of the upwelling along the coast of Arabia which is clearly marked in Fig. 3 by an area of reduced $\delta^{18}\text{O}$ amplitude.

Conclusions

The isotopic reconstruction of the last ice age for the northern Indian Ocean indicates a weaker south-west monsoon. This

would cause less precipitation over the Indian subcontinent, much of the Himalayas, the eastern and southern Tibetan uplands and Bangladesh which are drained by the Ganges, Brahmaputra, Irrawaddy and Salween rivers. This pattern is confirmed by continental evidence which shows that, at this time, the Persian Gulf region³⁶ and the Rajasthan³⁷ were dry, the Zagros Mountains in the north were drier than they are today³⁸ and strong loess storms occurred in the cold sand deserts of Inner Asia^{39–41}. This result is consistent with the widespread aridity in tropical Africa^{5,6,42,43} and dune activity in Arabia and northwestern India⁴¹. Continental aridity contrasted sharply with heavy oceanic precipitation south of 10° N, a pattern which supports the simulation of the tropical climate of the last ice age made with the GFDL general circulation model of the atmosphere⁴⁴. The oceanic reconstruction provides weak indications that the north-east monsoon was stronger than today in glacial conditions. The occurrence of strong north-east dry winds is supported by the large influx of pollen from the Irano-Turanian steppe of the north and north-east³⁰ and the higher percentage of quartz⁴⁵ in Arabian Sea sediments.

In the glacial world, the contrast between a weak south-west monsoon and a strong north-east monsoon is probably related to the environmental conditions prevailing over the Asian continent. In modern conditions, the central and southeastern Tibetan plateau which remains free of snow throughout the year, seems to accelerate the onset of the Asian monsoon and to increase its ultimate intensity because it must be rapidly heated during the spring in the Northern Hemisphere⁴⁶. During the last ice age, these mountains were covered with glaciers^{47,48}. This permanent ice coverage would have prevented any marked warming of the Asian continent at high altitude during the summer. Thus, the pressure difference between the Asian continent and the Indian Ocean would be smaller than today and the south-west monsoon weaker. By contrast, during winter, the presence of permanent ice would have helped to maintain a continent much cooler than today in the vicinity of a warm ocean, a mechanism that would have enhanced the north-east monsoon.

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Protochordate allorecognition is controlled by a MHC-like gene system

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Colonial tunicates, unlike vertebrates, undergo transplantation in nature. Rejection or acceptance between colonies of Botryllus is controlled by a single gene locus with multiple alleles. The same genetic region apparently maintains this polymorphism by preventing fertilization between gametes sharing alleles. The Botryllus histocompatibility system may reflect the original adaptive function of ancestral MHC genes.

In vertebrates, immune recognition of tissue antigens is responsible for rejection of transplanted allogeneic organs, tissues and cells¹. Prompt rejection of allotransplants by the cell-mediated immune system usually occurs when donor and host differ genetically at the major histocompatibility complex (MHC)^{2,3}. It appears that all vertebrate species may have MHC genes and the capacity for response to MHC-disparate antigens⁴, but the extent to which the MHC and adaptive immunity are found in invertebrate metazoans is unclear⁵. While vertebrate species exist normally as individuals which encounter foreign transplantation antigens only as a consequence of experimental transplantation, some members of invertebrate phyla allow the coexistence or fusion of individuals (or of groups of individuals, or of newly budded offspring to their parents) to form colonies. In fact, colonial forms are found in virtually all metazoan branches leading to the vertebrates. Recent studies have established the capacity for tissue transplant histocompatibility discrimination in both solitary and colonial members of diverse invertebrate animal phyla^{6,7}. Because of their phylogenetic position as the most primitive chordates, certain colonial tunicates provide a very interesting example of colony specificity, where fusion of neighbouring individuals or colonies is under strict genetic control⁸⁻¹⁰, and where, by contrast to the vertebrate situation, transplant rejection may be essential for survival. We set out to test the hypothesis that allorecognition in colonial tunicates is controlled by genes of an ancestral MHC gene complex⁷.

Botryllus colonies are usually clones of individuals (zooids) sharing a common vascular network and covered by a gelatinous tunic. Colonies grow by budding from a founder individual (oozoid), which arises by metamorphosis from a swimming tadpole-like larva. Production of new buds, resorption of old individuals, ovulation and sperm release occur synchronously for all individuals in a colony and are apparently controlled by blood elements^{11,12}. In the natural environment, ovulation occurs immediately on 'takeover' by a new generation of buds. Eggs are fertilized by sperm which enter from the surrounding seawater when the siphons of the new buds open. Embryonic development is phase-locked to the budding cycle, so that developed tadpoles hatch and swim away shortly before their parent zooids are resorbed and replaced by the next generation of buds. The hatched tadpoles, in turn, settle and metamorphose to become oozoids, and begin budding to produce new colonies.

Studies of the genetic control of intercolony fusibility by Watanabe and Oka⁹ performed with cut pieces from grown *Botryllus* colonies have established (for *Botryllus primigenus*) that each colony gathered from the marine environment is heterozygotic at a single fusibility gene locus represented in natural populations by many codominant alleles, and that two colonies must share one allele in order to fuse^{9,10}. The fusion

itself involves apposition of two colonies, dissolution of the intervening tunic, contact between extracorporeal blood vessel termini (ampullae) and fusion (or nonfusion) of the contacted blood vessels to form a multi-individual chimaeric colony with a common blood circulation^{13,14}. In this study we have developed a rapid fusion microassay which uses pairs of newly metamorphosed individual oozoids from *in vivo* or *in vitro*¹² crosses. *Botryllus* oozoids possess eight vascular microampullae which are involved in fusion or rejection reactions identical in appearance and time course to those described for ampullae from growing edges of adult colonies (refs 13, 14; Fig. 1). Our results show that allorecognition and control of fertilization, two phenomena characteristic of the murine MHC-bearing chromosome 17, are present as linked characters in two species of the colonial tunicate *Botryllus*.

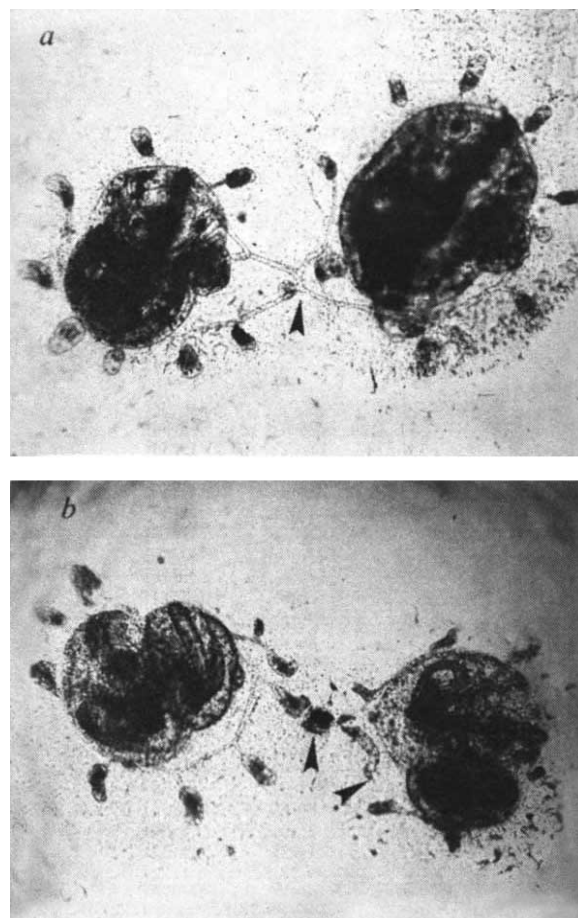


Fig. 1 *Botryllus* oozoids paired for fusibility testing. *a*, Fused oozoids; arrow points to new vascular connection. *b*, Rejected oozoids; arrow points to necrotic zone and detached vascular ampullae. $\times 240$.

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Colony fusion is controlled by a single, highly polymorphic genetic region

To examine the suitability of this microfusion assay for genetic studies and to determine whether genetic control of fusibility in Monterey *Botryllus* is similar to that described for *Botryllus schlosseri*¹⁰, we first examined fusibility frequencies for pairs of oozoids hatched from colonies fertilized in the natural environment. If colonies are heterozygotic at one locus determining fusibility (that is, AB ; Fig. 2) at which there are many codominant alleles ($A, B, C, D, \dots$) and if they are fertilized by sperm carrying a large number of (unknown) fusibility alleles (designated x_1 , or x_2 , or $x_3 \dots y$), then 25% of random pairs of oozoids hatched from this wild-fertilized colony will be

Table 1 Fusion frequencies for a , oozoid pairs from single wild-fertilized *Botryllus* colonies, and b , oozoid pairs from single wild-fertilized *Botryllus* colonies from crowded locations

Colony no.			
<i>a</i>			
<i>Botryllus</i> sp.			
(Monterey)	<i>n</i>	Fusions (%)	Rejections (%)
1	32	17(53)	15(47)
2	25	11(44)	14(56)
3	14	6(43)	8(57)
4	35	15(43)	20(57)
5	18	5(28)	13(72)
6	25	10(40)	15(60)
7	44	23(52)	21(48)
8	38	20(53)	18(47)
9	28	15(54)	13(46)
10	28	15(54)	13(46)
11	53	29(55)	24(45)
12	8	6(75)	2(25)
13	19	10(53)	9(47)
Total	367	182(50)	185(50)

$P > 0.8^*$

<i>B. schlosseri</i>			
(Woods Hole)			
1	13	5(38)	8(62)
2	24	13(54)	11(46)
3	12	9(75)	3(25)
4	11	5(45)	6(55)
5	16	7(44)	9(56)
6	13	5(38)	8(62)
7	13	7(54)	6(46)
8	15	8(53)	7(47)
9	46	21(46)	25(54)
10	16	10(63)	6(37)
11	16	8(50)	8(50)
Total	195	98(50)	97(50)

$P > 0.5^*$

<i>b</i>			
<i>Botryllus</i> sp.			
(Monterey)	<i>n</i>	Fusions (%)	Rejections (%)
1	11	7(64)	4(36)
2	46	31(67)	15(33)
3	11	8(73)	3(27)
4	19	12(63)	7(37)
5	6	4(67)	2(37)
6	7	5(71)	2(29)
7	78	47(60)	31(40)
8	22	14(64)	8(36)
9	7	6(86)	1(14)
10	26	17(65)	9(35)
11	18	13(72)	5(28)
12	39	30(77)	9(23)
13	14	12(86)	2(14)
14	37	20(54)	17(46)
15	28	13(46)	15(54)
16	10	5(50)	5(50)
17	29	19(66)	10(34)
18	40	32(80)	8(20)
19	12	8(66)	4(33)
20	6	3(50)	3(50)
21	15	11(73)	4(27)
22	37	29(78)	8(22)
23	9	6(67)	3(33)
Total	527	352(67)	175(33)

composed of Ax_1 , or Ax_2 , or $Ax_3 \dots Ay$ (designated $Ax \dots y$), all of which will fuse with each other due to sharing of the A allele; another 25% of random pairs will be composed of Bx_1 , or Bx_2 , or $Bx_3 \dots By$ (designated $Bx \dots y$), all of which will fuse with each other due to sharing of the B allele; and 50% of the random pairs will be $Ax \dots y$ paired with $Bx \dots y$, none of which will fuse with each other due to non-sharing of A or B alleles, and non-sharing of the $x \dots y$ alleles if a large number of distinct sperm donors ($x \dots y$) are involved in the fertilization.

Colonies of *Botryllus* were collected from the Monterey Marina (designated *Botryllus* sp.) or from the Green Pond at Falmouth or the Eel Pond at Woods Hole, Massachusetts (*B. schlosseri*). Embryonic development of wild-fertilized eggs was completed in the laboratory, and progeny oozoids were paired immediately after hatching and scored for fusion or rejection 48 h later. In both studies, colonies gathered from scattered locations consistently yielded oozoid progeny with a fusion frequency of ~50% (Table 1). This system thus gives results which parallel those obtained by other methods^{9,10}.

If fusibility in Monterey *Botryllus* is controlled by a single polymorphic gene locus, and one shared allele is required for fusion, then crosses between any two colonies collected from different locations (represented by $AB \times CD$) will provide progeny oozoids which are AC , AD , BC and BD at the fusion locus, and 75% of random pairs of the hybrid oozoids should fuse; this was the case with crosses at *Botryllus* colonies from both locations (Table 2a). Late in the growing season (February), many *Botryllus* colonies are crowded together and fertilizing sperm come predominantly from one or a few neighbouring colonies. One would thus expect newly hatched oozoids from these matings to give fusibility frequencies of 50–75%; in fact, fusion frequencies of 60–80% were obtained in such cases (Table 1b).

Fusibility alleles are not homozygous lethals

In no case did sampled colonies from East and West coast collecting sites give fusibility frequencies significantly above

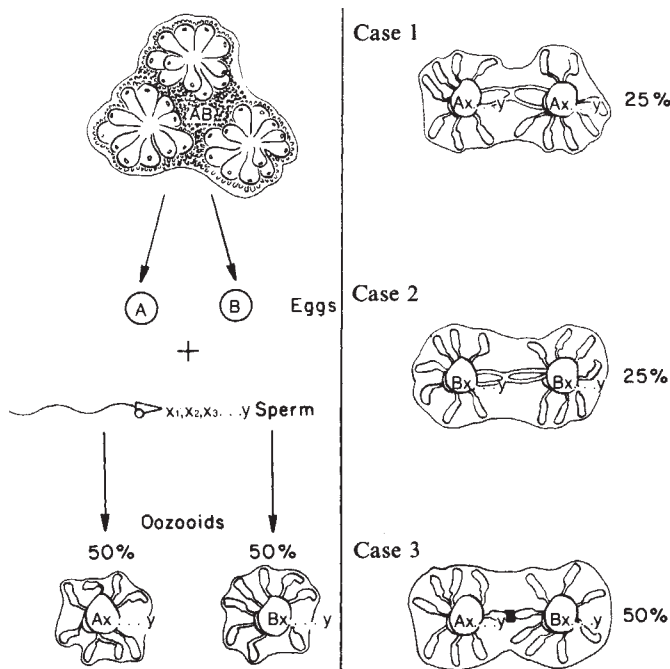


Fig. 2 Testing of the one locus, multiple-allele model for genetic control of colony specificity using the oozoid microassay. Paired oozoids for any crossed *Botryllus* colony will show at least 50% fusions, regardless of the number of alleles involved in the fertilization (above). As the number of sperm alleles participating in the fertilization is decreased, the probability that any two pairs will share a paternal allele increases, and fusibility frequency approaches the 75% frequency for a defined cross ($AB \times CD$, see text and Table 2).

*After data from different colonies in each of the studies were found to be homogeneous, χ^2 was computed and the P value determined. χ^2 for each study was calculated from individual samples as $\chi^2 = -2 \sum \ln P$, where P is the P value corresponding to the value of χ^2 (for goodness of fit) for individual experiments.

50–75% (Table 1), so we conclude that homozygotes at the fusibility locus may be rare or nonexistent in natural populations of both North American *Botryllus* species, as may be the case for *B. primigenus* in Japan⁹. Although they are hermaphroditic, colonies of *B. primigenus* have been claimed to be incapable of self-fertilization⁹ despite simultaneous ovulation and sperm release. In the two *Botryllus* species studied here, ovulation and fertilization by exogenous sperm occur before endogenous sperm release (protogyny), thus limiting self-fertilization in natural conditions (ref. 15, and V.L.S., unpublished results). When protogyny is circumvented by crossing between separated pieces of the same colony which have been phase-separated by temperature, self-fertilization does occur, but a large proportion of the resulting embryos undergo developmental arrest, or fail to complete metamorphosis after hatching¹⁵.

To test whether embryonic maldevelopment which occurs in the progeny of self-crosses in *B. schlosseri*¹⁵ and Monterey *Botryllus* (V.L.S. and J.M.S., unpublished) replaces the self-sterility operating in *B. primigenus*, we isolated seven colonies, allowed them to self-cross ($AB \times AB$) and counted the fusion frequencies of the few surviving oozoids. If homozygotes (AA and BB) fail to develop, all the survivors should be identical (AB) and the fusion frequency should be 100%. However, none of the self-crosses gave this result (Table 2b). Thus, although it is possible that the fusion locus causes late maturation and differentiation effects in homozygotes¹⁵, the fusibility locus cannot be the major determinant in inbreeding depression. It was, therefore, necessary to find another explanation for the puzzling absence of homozygotes in the two *Botryllus* populations studied.

Fusibility locus affects self-fertilization by sperm–egg incompatibility

We have observed that in Monterey *Botryllus*, as in *B. schlosseri*¹⁵, self-fertilizations do not occur synchronously and immediately after ovulation as do cross-fertilizations; in colonies isolated for selfing, the cleavages begin at scattered times and some eggs are never fertilized. We therefore designed experiments to test the hypothesis that there is a gametic barrier to fertilization which breaks down in time to allow self-fertilization in laboratory conditions of isolation. At Woods Hole, 18 colonies of *B. schlosseri* were isolated, 5 of which were early ovulating and fertilized by exogenous sperm brought in from the collection site. In these 5 colonies fertilizations were synchronous, and gave large hatches of oozoids whose fusibility frequency was ~50%. The other 13 late-ovulating colonies were placed *in vitro* in conditions allowing self-fertilization. In these conditions early embryonic cleavages were scattered in colonies whose eggs were ovulated after 2 days of isolation, and 7/13 of these colonies showed delays of 2–4 days after ovulation before the first fertilizations occurred. This occurred despite sperm-producing testes being present in each colony from 1 day after ovulation onwards (data not shown). It seems likely that *Botryllus* eggs resist fertilization by sperm from the same colony for varying periods of time after ovulation.

To extend this observation to the Monterey species, paired colonies at ovulation were divided in half, with one of the subcolonies (A_1 and B_1) from each pair placed in isolation and the others (A_2 and B_2) placed together. In all experiments, cross-fertilization (A_2/B_2) occurred between the paired pieces 1–2 days before self-fertilization (A_1/A_1 and B_1/B_1) occurred in the isolated controls (data not shown), indicating that sperm-producing testes and fertilization-competent eggs are present in both colonies at a time when self-fertilization cannot occur.

To determine whether this barrier to self-fertilization is controlled by the fusibility locus, colonies from the same algal blade were crossed. Our sampling data for colonies with contacted borders indicate that, in these conditions, >20% of such defined crosses should be between sibling colonies sharing at least one fusibility allele. We expected for such semiallogeneic crosses ($AB \times BC$) that sperm carrying the shared allele (B)

Table 2 Fusion frequencies for F_1 oozoid pairs from defined crosses

a, Cross-fertilizations				
Colony no.				
<i>Botryllus</i> sp. (Monterey)	<i>n</i>	Fusions(%)	Rejections(%)	
1	10	7(70)	3(30)	
2	17	12(71)	5(29)	
3	35	23(66)	12(34)	
4	14	10(71)	4(29)	
5	6	4(67)	2(33)	
6	5	3(60)	2(40)	
7	29	22(76)	7(24)	
8	52	37(71)	15(29)	
9	9	8(89)	1(11)	
10	7	6(86)	1(14)	
11	4	3(75)	1(25)	
12	17	13(76)	4(24)	
13	7	5(71)	2(29)	
14	13	10(77)	3(23)	
15	26	20(77)	6(23)	
16	6	4(67)	2(33)	
17	22	16(73)	6(27)	
18	8	7(88)	1(12)	
19	9	8(89)	1(11)	
20	67	49(73)	18(27)	
Total	363	267(74)	96(26)	$P = 0.9^*$
<i>B. schlosseri</i> (Woods Hole)				
1	8	6(75)	2(25)	
2	29	18(62)	11(38)	
3	20	13(65)	7(35)	
4	5	4(80)	1(20)	
Total	62	41(67)	21(33)	$P = 0.5^*$
b, Self-fertilizations				
<i>Botryllus</i> sp. (Monterey)	<i>n</i>	Fusions(%)	Rejections(%)	
1	8	6(75)	2(25)	
2	15	13(87)	2(13)	
3	5	4(80)	1(20)	
4	4	3(75)	1(25)	
Total	32	26(81)	6(19)	
<i>B. schlosseri</i> (Woods Hole)				
1	5	4(80)	1(20)	
2	4	3(75)	1(25)	
3	3	2(67)	1(33)	
Total	12	9(75)	3(25)	

When two colonies are crossed ($AB \times CD$), not only is the maternal allele shared in 50% of the oozoid pairs (above), but the paternal allele is also shared in 50% of the pairs (half of which are pairs not sharing a maternal allele); thus the fusibility frequency for these oozoids is 75% (Table 2a). All oozoid pairs hatched from a self-fertilization (except the 1/8 which are composed of two homozygotes for opposite alleles— AA to BB) share at least one allele; thus 87.5% will fuse (Table 2b).

*Data were analysed as described in Table 1 legend.

Table 3 Fusion frequencies for oozoid pairs from colonies crossed with potential siblings

Colony no.	<i>n</i>	Fusions(%)	Rejections(%)
1	43	35(81)	8(19)
2	17	13(76)	4(24)
3	22		0
4	11	6(55)	5(45)
5	25	17(68)	8(32)
6	30	20(67)	10(33)
7	6		0
8	18	12(67)	6(33)
9	8		0
10	8	6(75)	2(25)
11	10	8(80)	2(20)
12	14	9(64)	5(36)
13	18		0
14	13	10(77)	3(23)
15a \ Reciprocal	19		0
15b } crosses	113	19(100)	0
16	6	5(83)	1(17)
17	14		0
Total	281 (195)	141(72)	54(28)
		86(100)	

For all data, $\chi^2_1 = 17.58$ ($P = 0.005$); thus we reject the null hypothesis that no gametic barrier to fertilizations by sperm carrying the shared allele exists between crossed sibling colonies (see text).

Table 4 Fusibility frequencies for F_2 oozoids

Cross	n	Fusions	Rejections	% Fusions
1	27	27	—	100
2a	13	13	—	100
2b	11	11	—	100
3a	49	49	—	100
3b	40	40	—	100
4	10	9	1	90
6	47	47	—	100
7	7	7	—	100
8	29	20	9	69
9	21	18	3	86

From an $AB \times CD$ defined cross, four classes of offspring, present in equal numbers, are expected: AC , AD , BC and BD . Random crossings of the grown progeny colonies from such a cross were expected to be of three kinds: (1) one-half should be crosses where one fusibility allele is shared, and only sperm carrying the non-shared allele can fertilize the eggs, giving offspring which are 100% fusible with one another; (2) one-quarter should be crosses where both fusibility alleles are shared, giving oozoid progeny which are 87.5% fusible (Table 2b) and small hatches because fertilization is delayed for 24–48 h until the gametic barrier has broken down; (3) one-quarter should be crosses where no alleles are shared, giving the expected 7.5% fusible progeny (Table 2a). 'a' and 'b' notations denote reciprocal crossings between the same two colonies.

should not fertilize any of the eggs of the crossing partner, thus allowing all eggs to be fertilized by the sperm carrying the nonshared allele (all by A or all by C , depending on which colony serves as a sperm donor). All offspring from such a cross should share a fusibility allele. Table 3 shows the results of fusion analysis of oozoids from the 17 crosses performed in this study. Although 11 of the 17 crosses produced oozoids whose fusion frequencies were close to those expected for cross-fertilizations (75%), 6/17 gave frequencies of 100% (Table 3). The above data are most consistent with a model for self-sterility in which the haploid expression of fusibility allele patterns by sperm is recognized by the diploid, maternally derived egg envelopes⁹, resulting in selective fertilization by sperm bearing new fusibility alleles.

To test these findings further, sibling *B. schlosseri* colonies, grown from the F_1 oozoid progeny of a defined cross ($AB \times CD$) were crossed with one another. Two of the eight crosses performed produced F_2 oozoids whose pairs gave fusibility frequencies close to 75% (Table 4). This is consistent with the expectation that 25% of such crosses will involve no shared alleles (this is the proportion of the grown colonies which would not have fused as oozoids; Table 2a). Paired oozoids hatched from all but one of the remaining six crosses (a frequency consistent with that proportion of defined-cross F_1 oozoids which fuse; Table 2a) gave fusibility frequencies of 100%. Fertilizations between colonies sharing both fusibility alleles (which should comprise 25% of the crosses between sibling colonies in this experiment) can occur only after the selfing barrier has broken down, after which scattered, asynchronous fertilizations lead to small hatches (Table 2b). The expected fusibility frequency for such oozoids is 87.5% (Table 2b). Very small hatches, giving fusibility frequencies of 90%, 100%, and

100% respectively (Table 4), resulted from three of the eight crosses in this experiment (Table 4). On the other hand, fertilizations between colonies sharing one fusibility allele, a group which should comprise one-half of the crossings between F_1 progeny of a defined cross, should yield oozoids whose pairs give 100% fusions, as was found in three of the eight defined crosses between sibling colonies shown in Table 4. These data support our hypothesis for genetic control of colony specificity tested in other experiments (Tables 1, 2) and, with the results shown in Table 3, confirm the prediction by Watanabe and Oka⁹ of a gametic barrier to fertilization by sperm carrying shared fusibility alleles.

Discussion

The *Botryllus* fusibility gene locus shows the same degree of polymorphism as the genes of the vertebrate MHC¹⁴ and the self-incompatibility systems of plants^{16,17}. Because *Botryllus* histocompatibility genes control both somatic tissue recognition and response, as does the vertebrate MHC, and gamete behaviour at fertilization, as does the S_s system in the angiosperms, our findings support hypotheses^{16,18,19} that vertebrate histocompatibility genes evolved from gametic self-nonself recognition systems which prevented self-fertilization in hermaphroditic organisms. It seems likely that self-nonself discrimination in *Botryllus* may represent the evolutionary precursor(s) to the MHC⁷. Polymorphism at the *Botryllus* and vertebrate loci may be maintained through linkage of histocompatibility genes with elements which enforce the heterozygous condition through control of fertilization^{20–22} or development^{22–24}.

Despite the similarities between the vertebrate MHC and the *Botryllus* fusion systems, several major differences have yet to be reconciled. For example, fusion is allowed between ampullae of *Botryllus* colonies sharing one allele. In vertebrate allorecognition, by contrast, lymphocyte clones recognize and eliminate cells carrying non-shared histocompatibility antigens, even in the presence of one shared allele. However, events subsequent to a fusion between semiallogeneic *Botryllus* oozoids or colonies (that is AB fused to BC) frequently lead to the complete resorption of one member of a fused oozoid pair (V.L.S., in preparation) or to the disappearance of germ cells of one of two fused colonies (A. Sabbadin, personal communication). Further studies of these delayed (or induced) responses to contact between semiallogeneic cells may therefore disclose even more similarities between both the native immune reactivities (for example colony specificity and the natural killer cell reactivities of mammals) and the induced allogeneic recognition responses in these two chordate systems. For these purposes, it will be important to determine whether *Botryllus* allorecognition is a capacity possessed by all cells, or only by ampullar and blood cells^{13,14}.

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Expression of human immune interferon cDNA in *E. coli* and monkey cells

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A cDNA sequence coding for human immune interferon (IFN- γ) has been identified in a cDNA library prepared from gel-fractionated IFN- γ mRNA. The DNA sequence codes for a polypeptide of 166 amino acids, 20 of which could constitute a signal peptide. The polypeptide produced through expression of this DNA sequence in Escherichia coli or cultured monkey cells had properties characteristic of authentic human IFN- γ .

INTERFERONS (IFNs) are secreted proteins which induce an antiviral state in their target cells, and which have immunomodulatory and antitumour properties¹. On the basis of antigenicity and biological and chemical properties, human interferons have been grouped into three major classes: IFN- α (leukocyte), IFN- β (fibroblast) and IFN- γ (immune)². Considerable information has accumulated on the structures and properties of the virus-induced acid-stable interferons (IFN- α and - β). These have been purified to homogeneity and partial amino acid sequences determined³⁻⁷. Analyses of cloned cDNA and gene sequences for IFN- β (refs 8-10) and the IFN- α multigene family¹¹⁻¹⁶ have permitted the deduction of the complete amino acid sequences of many of these interferons. In addition, efficient synthesis of IFN- β (ref. 10) and several IFN- α s^{13,16} in *Escherichia coli* and IFN- α 1 in yeast¹⁷ have now made possible the purification of large quantities of these proteins in biologically active form.

Much less data are available concerning the structure and properties of IFN- γ . IFN- γ is generally produced in cultures of lymphocytes exposed to various mitogenic stimuli, is acid labile and does not cross-react with antisera prepared against IFN- α or IFN- β (ref. 1). Molecular weights ranging from 35,000 to 70,000 have been reported for IFN- γ (refs 18-21). Recently, a quite extensive, but still partial, purification of IFN- γ has been reported²⁰, and IFN- γ mRNA has been isolated and translated in *Xenopus laevis* oocytes^{22,23}. A broad range of biological activities have been attributed to IFN- γ , including potentiation of the antiviral activities of IFN- α and - β (ref. 24), and it differs from these interferons in its virus and cell specificities and in the antiviral mechanisms it induces²⁵. However, *in vitro* studies performed with crude preparations suggest that the primary function of IFN- γ may be as an immunoregulatory agent²⁶. The antiproliferative effect of IFN- γ on transformed cells has been reported to be 10-100-fold greater than that of IFN- α or - β (refs 25, 27), suggesting a potential use in the treatment of neoplasia. Indeed, murine IFN- γ preparations have been shown to have significant antitumour activity against mouse sarcomas²⁸. However, the poor availability of purified IFN- γ has precluded the unambiguous determination of its physicochemical and biological properties.

We describe here the first isolation and characterization of a recombinant plasmid containing a cDNA sequence coding for human IFN- γ . Expression of this sequence in *E. coli* and cultured monkey cells gives rise to a polypeptide having the properties of authentic human IFN- γ .

Construction and identification of bacterial clones containing induced cDNA sequences

Human peripheral blood lymphocytes (PBLs) from healthy donors were stimulated to produce IFN- γ by treatment with staphylococcal enterotoxin B (SEB) and desacetylthymosin α_1 as described by Svedersky *et al.*²⁹. Cell cultures from individual

donors were collected 48 h after induction and used to prepare polyadenylated RNA^{30,31} which was fractionated by electrophoresis through denaturing agarose gels^{32,33}. Interferon mRNA activity was determined by injecting aliquots of each fraction into *X. laevis* oocytes³⁴⁻³⁷ and after 24 h assaying the incubation medium for antiviral activity^{36,37}. One peak of activity was consistently observed which co-migrated with 18S RNA (Fig. 1). Using different procedures for sucrose density gradient centrifugation, Wallace *et al.*²² and Taniguchi *et al.*²³ have reported single peaks of IFN- γ mRNA activity sedimenting at 18S and 15S respectively. RNA from our most active 18S gel fraction (600 units ml⁻¹ when 0.5 μ g was injected into 15 oocytes at a concentration of 0.5 μ g μ l⁻¹) was used to prepare double-stranded cDNA by standard procedures^{38,39}. The cDNA was fractionated according to size and material longer than 800 base pairs (bp) was extended with deoxy(C) residues, annealed to deoxy(G)-tailed *Pst*I-cleaved pBR322 and used to transform *E. coli* K-12 strain 294 (ref. 40) as described previously^{13,19}.

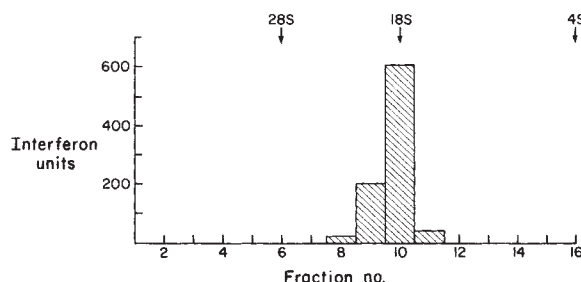


Fig. 1 Gel electrophoresis of induced lymphocyte polyadenylated RNA. Peripheral blood lymphocytes (PBLs) derived from individual human donors by lymphoporesis were purified by centrifugation on a Ficoll-Hypaque gradient and induced for IFN- γ production with staphylococcal enterotoxin B (1 μ g ml⁻¹) and desacetylthymosin- α_1 (0.1 μ g ml⁻¹)²⁹. Total RNA was extracted³⁰ from PBL cultures and poly(A)-containing RNA was purified by oligo(dT) cellulose chromatography³¹. 200 μ g of mRNA was fractionated by electrophoresis through a denaturing agarose gel (1.75% agarose, 0.025 M sodium citrate (pH 3.8) and 6 M urea) for 7 h at 25 mA and 4°C (refs 32, 33). The slab gel was sectioned with a razor blade and individual slices were melted at 70°C and extracted twice with phenol and once with chloroform. Fractions were precipitated with ethanol and subsequently assayed for IFN- γ mRNA by injection into *X. laevis* oocytes³⁴⁻³⁷ at a concentration of 0.5 μ g μ l⁻¹. After 24 h incubation at room temperature the antiviral activity of the translation products in the incubation medium was determined by the cytopathic effect (CPE) inhibition assay using vesicular stomatitis virus or encephalomyocarditis virus on WISH (human amnion) cells¹. All IFN- γ units are expressed with reference to the NIH IFN- α standard (G-023-901-527), because no internationally accepted IFN- γ standard was available. In this assay system 0.2 units of the standard results in a 50% reduction in cytopathogenic effect. A single peak of activity was observed which co-migrated with 18S ribosomal RNA. The positions of ribosomal RNA markers which were electrophoresed in an adjacent lane and visualized by ethidium bromide staining are labelled above the activity profile. The activity seems to be due to IFN- γ , as no activity was observed when the same fractions were assayed on a bovine cell line (MDBK) which is not protected by IFN- γ (ref. 29). Both IFN- α activity and IFN- β activity would have been easily detectable with the MDBK assay¹⁰.

We screened 8,300 bacterial clones from the cDNA library for the presence of specifically induced cDNA sequences by the following procedure. Two copies of the colony library were grown up on nitrocellulose filters and DNA was fixed to the filters for *in situ* colony hybridization^{41,42}. One set of filters was hybridized with a ³²P-labelled cDNA probe prepared using the same 18S gel-fractionated mRNA (induced) used to prepare the colony library. The second set of filters was hybridized with ³²P-labelled cDNA prepared using 18S gel-fractionated poly(A) RNA isolated from unstimulated PBLs. The hybridization intensity of each colony with the two probes was compared, as IFN- γ sequences should only hybridize with the induced probe. 124 colonies hybridized with the induced probe but undetectably or weakly with the uninduced probe (see Fig. 2). Plasmid DNA was isolated from each of these colonies⁴³, bound to nitrocellulose filters⁴⁴ and hybridized with the same induced and uninduced probes. DNA from 22 colonies hybridized specifically with the induced probe in this rescreening.

The cDNA inserts from several of these 'induced' colonies were mapped using restriction endonucleases and found to contain common *DdeI*, *HincII* and *RsaI* sites (Fig. 3a). A ³²P-labelled DNA probe was prepared⁴⁵ from one of the plasmids using two internal *DdeI* fragments (130 and 600 bp) and

hybridized to DNA from the 124 colonies selected in the first round of screening. Only the 22 induced clones were found to hybridize with this probe, establishing their relatedness. Subsequent rescreening^{41,42} of the cDNA library with this same probe revealed that approximately 1 in 120 *E. coli* clones contained related sequences. Digestion of plasmid DNA from these clones with *PstI* showed that clone 69 contained the largest cDNA sequence (~1,250 bp).

Sequence analysis of the cDNA insert of plasmid p69

The complete nucleotide sequence of the *PstI* insert from plasmid p69 was determined by the Maxam-Gilbert chemical procedure⁴⁶ and by the dideoxynucleotide chain termination method⁴⁷ after subcloning fragments into the M13 vector mp7 (ref. 48). The presence of 19 consecutive A residues corresponding to the 3' poly(A) terminus of the mRNA permitted us to orient the 1,200 nucleotide long sequence as shown in Fig. 3b. As IFN- γ mRNA co-migrates with 18S ribosomal RNA (>2,000 nucleotides) on denaturing agarose gels, it was important to see whether the 1,200 bp insert of p69 represented a full-length copy of its respective mRNA. To measure the length of this mRNA the synthetic tridecanucleotide dTCGTTTCCGAGAG, complementary to nucleotides 98-110 of the p69 insert (Fig. 3b), was chemically synthesized⁴⁹, 5' ³²P-labelled and used to prime the reverse transcription of induced lymphocyte poly(A) RNA into single-stranded ³²P-cDNA. The cDNA product was sized on a polyacrylamide-urea gel⁴⁶ and found to be 125-130 nucleotides long (unpublished results), indicating that the cDNA insert of p69 lacks only about 15-20 nucleotides of 5' mRNA sequence. Induced poly(A) mRNA was also fractionated on a denaturing agarose gel^{33,34}, transferred to nitrocellulose paper⁵⁰ and hybridized with ³²P-labelled cDNA insert from p69. A single hybridizing band, with the same mobility as 18S rRNA, was observed. Therefore, the mRNA corresponding to the p69 cDNA insert co-migrates with IFN- γ mRNA activity.

The DNA sequence of the p69 cDNA insert contains a single large open reading frame, beginning with the ATG codon at nucleotides 110-112 from the 5' end. This ATG is followed, 166 codons later, by a TAA termination triplet at nucleotides 608-610. This ATG is the first one encountered, consistent with the observation that the first ATG codon from the 5' end of mRNA usually serves as the site of translation initiation in eukaryotes⁵¹. The 3'-untranslated region of 587 nucleotides contains the hexanucleotide AATAAA (position 1,173-1,178) which precedes the site of polyadenylation in many eukaryotic mRNAs⁵². The 20 N-terminal amino acids encoded by the cDNA insert of p69 may constitute a signal peptide which is involved in the secretion of the mature polypeptide from lymphocytes. This putative signal sequence is strongly hydrophobic, having features common to eukaryotic secretory protein signal peptides in general^{53,54} and more specifically to the IFN- α s for which DNA sequences have been determined¹¹⁻¹⁶. It has the same potential cleavage site (Gly-Cys) that is likely to be the signal peptide cleavage sequence common to all IFN- α s¹⁴. Furthermore, the three amino acids (Ser-Leu-Gly) preceding this potential cleavage site are identical to those encoded by 10 of the 11 IFN- α genes sequenced to date¹¹⁻¹⁶.

The only other homology between this potential IFN- γ sequence and IFN- α or IFN- β covering more than three consecutive amino acids occurs at amino acids 15-18 (Lys-Lys-Tyr-Phe). These same four amino acids are found at positions 121-124 of two IFN- α s (IFN- α D and - α F; ref. 14). The 146 amino acid polypeptide estimated to constitute the mature protein has a calculated molecular weight of 17,110, much smaller than the molecular weights reported previously for IFN- γ (refs 18-21). The reasons for this discrepancy are not clear, but it should be noted that the molecular weights reported previously represent the size of the undenatured active species, which could exist as a multimer of individual subunits. Addi-

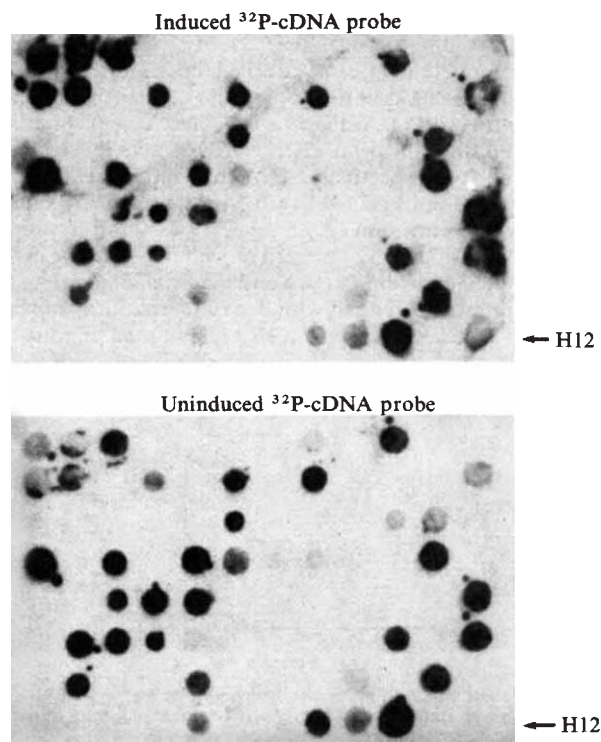


Fig. 2 Hybridization patterns of 96 colonies containing cloned cDNAs with induced and uninduced ³²P-labelled cDNA probes. Double-stranded cDNA was prepared by standard methods^{13,38,39} using 3 μ g of mRNA from gel fraction 10 (Fig. 1). The cDNA >800 bp (340 ng) was recovered by electroelution after fractionation on a 6% polyacrylamide gel. An aliquot (35 ng) of the cDNA was extended with deoxy(C) residues using terminal deoxynucleotidyl transferase⁷⁰, and annealed with 300 ng of the plasmid pBR322⁷¹ which had been similarly tailed with deoxy(G) residues at the *PstI* site. The annealed mixture was used to transform *E. coli* K-12 strain 294⁴⁰ and resultant tetracycline-resistant colonies were individually inoculated into wells of microtitre plates containing L broth⁶⁸ and 5 μ g ml⁻¹ tetracycline. Two copies of the cDNA library of 8,300 transformants were grown up on nitrocellulose filters and the DNA from each colony was fixed to the filter^{41,42}. ³²P-labelled cDNA probes were prepared from 18S size gel-fractionated mRNA from induced and uninduced PBL cultures. Oligo(dT)¹²⁻¹⁸ was used as a primer and reaction conditions have been previously described¹³. One set of 86 filters (96 colonies per filter) containing the entire cDNA library was hybridized⁷² to 2 $\times$ 10⁷ c.p.m. of the induced ³²P-cDNA. The duplicate set of filters was hybridized with 2 $\times$ 10⁷ c.p.m. of uninduced ³²P-cDNA. After 16 h at 45 $^{\circ}$ C, filters were washed extensively⁷² and exposed to X-ray film. The two filters shown here are representative of 86 such sets: approximately half the colonies hybridize with both probes, while half fail to hybridize with either probe, and ~1% hybridize with only the induced probe. An example of an 'induced' clone is labelled as H12.

Table 1 Characterization of IFN- γ produced by *E. coli* and monkey cells

Treatment	IFN- α standard	IFN- β standard	IFN- γ standard	Antiviral activity (U ml ⁻¹)	
				<i>E. coli</i> W3110/pIFN- γ trp48 extract	COS-7 cell/pSV γ 69 culture medium
Untreated	375	125	250	250	62.5
pH 2	375	125	<6	<12	<4
0.1% SDS	375	—	<4	<8	<4
Rabbit anti-IFN- α	<8	125	250	250	62.5
Rabbit anti-IFN- β	375	<8	187	250	62.5
Rabbit anti-IFN- γ	375	125	<4	<8	<4

An overnight culture of *E. coli* W3110/pIFN- γ trp48 in Luria broth⁶⁸ containing 5 μ g ml⁻¹ tetracycline was diluted 1:100 in M9 medium⁶⁸ containing 0.2% glucose, 0.5% casamino acids and 5 μ g ml⁻¹ tetracycline at a 1:100 dilution. Indole acrylic acid was added to a final concentration of 20 μ g ml⁻¹ when A_{550} was 0.1–0.2. Samples (10 ml) were collected by centrifugation at $A_{550} = 1.0$ ($\sim 3.5 \times 10^8$ cells ml⁻¹) and resuspended immediately in 1 ml phosphate-buffered saline containing 1 mg bovine serum albumin (PBS-BSA). Cells were opened by sonication and cleared of debris by centrifugation. The supernatants were stored at 4 °C until assay. The plasmid pSV γ 69 was introduced in COS-7 cells⁶² using a modification of the DEAE-dextran technique⁶⁹. Fresh monolayers of COS-7 cells in 6-cm diameter plates were transfected with 1 μ g of pSV γ 69 in 1.5 ml of Dulbecco's minimal essential medium (DMEM; Gibco), 200 μ g ml⁻¹ DEAE-dextran (500,000 molecular weight; Pharmacia), 0.05 M Tris-HCl (pH 7.5). After 16 h at 37 °C, the plates were washed twice with DMEM, and 1.5 ml of fresh DMEM supplemented with 10% fetal bovine serum, 2 mM glutamine, 50 μ g ml⁻¹ penicillin G and 50 mg ml⁻¹ streptomycin was added to each plate. The medium was replaced the following day with serum-free DMEM. Fresh serum-free medium was then added every day. The collected media were stored at 4 °C until assayed. IFN- α and IFN- β control samples were NIH reference standards. The IFN- γ control was partially purified ($\sim 10^6$ units per mg) from induced PBLs. The anti-IFN- α and - β were obtained from the National Institute of Allergy and Infectious Diseases. The anti-IFN- γ antisera was prepared using authentic IFN- γ (5–20% purity) purified from stimulated PBLs. Samples for antibody neutralizations were diluted, if necessary, then incubated for 2 h at 4 °C with 1:20 dilutions of rabbit anti-human leukocyte, fibroblast or immune interferon antisera. Samples were centrifuged for 3 min at 12,000g before assay. To test pH 2 stability, samples were adjusted to pH 2 by addition of 1 M HCl, incubated for 2 h at 4 °C and neutralized by addition of 1 M NaOH before assay. To test SDS sensitivity, samples were incubated with an equal volume of 0.2% SDS for 2 h at 4 °C before assay. The IFN assay was performed as described in Fig. 1 legend.

tionally, IFN- γ is glycosylated *in vivo*^{20,21,55}, which could affect molecular weight estimates. Consistent with this observation is the presence of two potential *N*-glycosylation sites⁵⁶ in the predicted protein sequence, at amino acids 28–30 (Asn-Gly-Thr) and 100–102 (Asn-Tyr-Ser). As the only two cysteines present in the deduced amino acid sequence occur at positions 1 and 3, the tertiary structure of the polypeptide is probably not dependent on intramolecular disulphide bonds. This is in agreement with the finding that, in contrast to IFN- α and IFN- β , disulphide linkages are not critical for the antiviral activity of IFN- γ (ref. 21). The mature protein sequence shown in Fig. 3b is quite basic in character, having 27 basic amino acids (Arg + Lys) and 19 acidic residues (Asp + Glu). This is consistent with the observation of Yip *et al.*²⁰ that IFN- γ has a high isoelectric point ($pI = 8.6$ – 8.7), whereas IFN- α and IFN- β have been shown to have distinctly lower pI s^{20,57}.

Synthesis of IFN- γ in *E. coli* and monkey cells

As IFN- γ has not been purified to homogeneity, nothing is known directly about its amino acid sequence. Therefore, the only direct means of proving that the cDNA sequence of p69 actually codes for IFN- γ is by demonstrating that the polypeptide obtained through expression of this cloned gene has the properties of natural IFN- γ . Two independent expression systems involving *E. coli* and monkey cells were used for this purpose. The *E. coli* system was selected because it has been used to produce biologically active IFN- α s and IFN- β (refs 10, 13). The mammalian cell system was chosen because, unlike *E. coli*, it should be able to perform specific processing (for example, glycosylation) which might be required for the expression of a biologically active product.

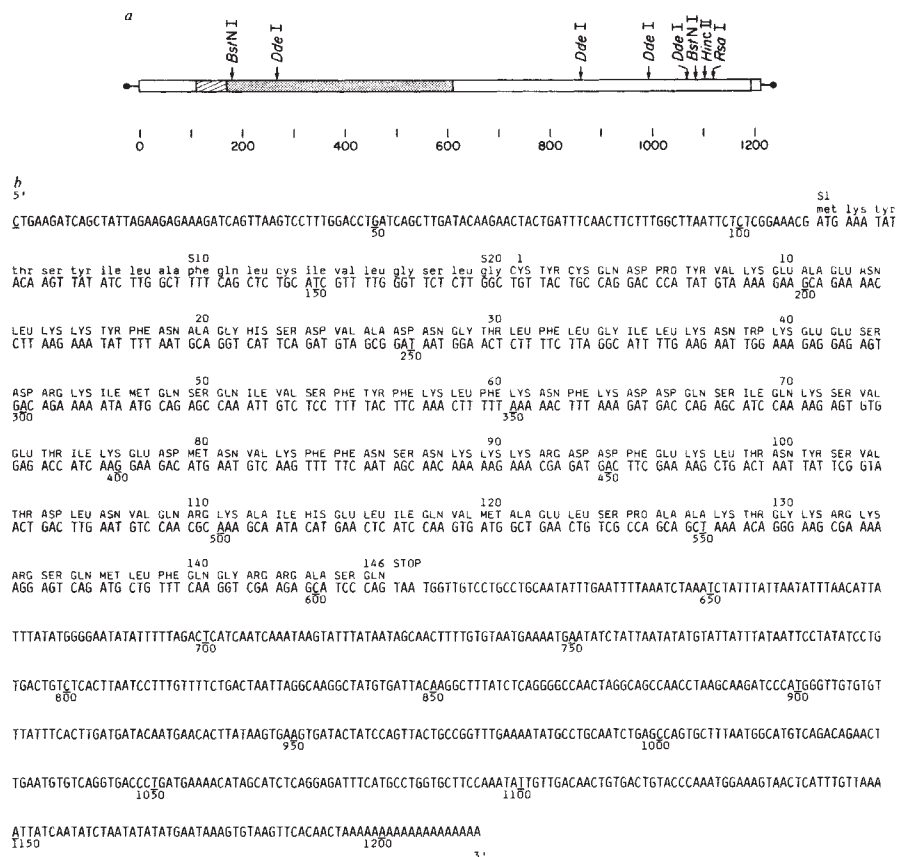


Fig. 3 a, Restriction endonuclease map of the cDNA insert of p69. The cDNA insert is bounded by *Pst*I sites (dots at both ends) and poly(dG-dC) tails (single lines). The number and size of fragments produced by restriction nuclease cleavage were estimated by electrophoresis through 6% polyacrylamide gels. Positions of sites were confirmed by nucleic acid sequencing (presented below). The shaded region indicates the coding sequences of the putative mature protein, the cross-hatched region represents the putative 20 residue signal peptide coding sequence, and the open regions show the 3' and 5' noncoding sequences. b, Nucleotide sequence and deduced amino acid sequence of plasmid p69 cDNA insert. The putative signal sequence is represented by the residues labelled S1 to S20. The entire sequence was determined by both the dideoxynucleotide chain termination method⁴⁷ after subcloning fragments into the M13 vector mp7⁴⁸ and the chemical method of Maxam and Gilbert⁴⁶. Numbers above each line refer to amino acid position (S refers to presumed signal peptide) and numbers below each line to nucleotide position.

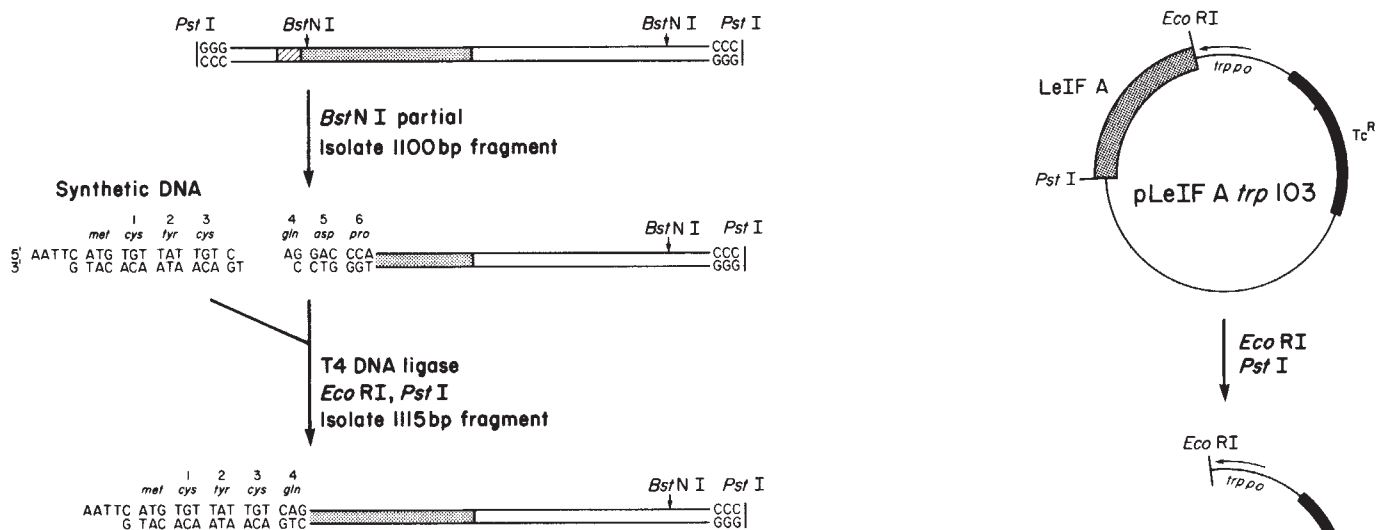


Fig. 4 Construction of a plasmid coding for the direct synthesis of mature IFN- γ in *E. coli*. 5 μ g of the 1,250 bp *Pst*I insert of plasmid p69 were isolated and partially digested with 3 units of *Bst*NI (Bethesda Research Laboratories) for 15 min at 37 °C and the reaction products resolved on a 6% polyacrylamide gel. Approximately 0.5 μ g of the desired 1,100 bp *Bst*NI-*Pst*I fragment was recovered by electroelution. The two indicated deoxyoligonucleotides, 5'-dAATTCATGTGTTATTGTC and 5'-dTGACAATAACACATG, were synthesized by the phosphotriester method⁴⁹ and phosphorylated as follows. 100 pmol of each deoxyoligonucleotide were combined in 30 μ l of 60 mM Tris-HCl (pH 8), 10 mM MgCl₂, 15 mM β -mercaptoethanol and 240 μ Ci [γ -³²P]ATP (Amersham, 5,000 Ci mmol⁻¹). 12 units of T4 polynucleotide kinase were added and the reaction allowed to proceed at 37 °C for 30 min. 1 μ l of 10 mM ATP was added and the reaction allowed to proceed for an additional 20 min. After phenol/CHCl₃ extraction, the oligomers were combined with 0.25 μ g of the *Bst*NI-*Pst*I, 1,000 bp fragment and ethanol precipitated. These fragments were ligated at 20 °C for 2 h in 30 μ l of 20 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 10 mM dithiothreitol, 0.5 mM ATP and 10 units T4 DNA ligase. The mixture was digested for 1 h with 30 units of *Pst*I and 30 units of *Eco*RI (to eliminate polymerization through ligation of cohesive termini) and electrophoresed on a 6% polyacrylamide gel. The 1,115 bp product (110,000 c.p.m.) was recovered by electroelution. The plasmid pLeIFAtmp103 is a derivative of the plasmid pLeIFA25 (ref. 13) in which the *Eco*RI site distal to the LeIF A gene has been removed. 3 μ g of pLeIFAtmp103 were digested with 20 units of *Eco*RI and 20 units of *Pst*I for 90 min at 37 °C and electrophoresed on a 6% polyacrylamide gel. The large (~3,900 bp) vector fragment was recovered by electroelution and the 1,115 bp *Eco*RI-*Pst*I IFN- γ DNA fragment ligated into 0.15 μ g of this prepared vector. Transformation of *E. coli* K-12 strain 294⁴⁰ gave 120 tetracycline-resistant colonies. Plasmid DNA was prepared from 60 of these transformants and digested with *Eco*RI and *Pst*I. Three of these plasmids contained the desired 1,115 bp *Eco*RI-*Pst*I fragment. DNA sequence analysis verified that these plasmids had the desired nucleotide sequence at the junctions between the *trp* promoter, synthetic DNA and cDNA. One of these plasmids, pIFN- γ trp48, was chosen for additional study.

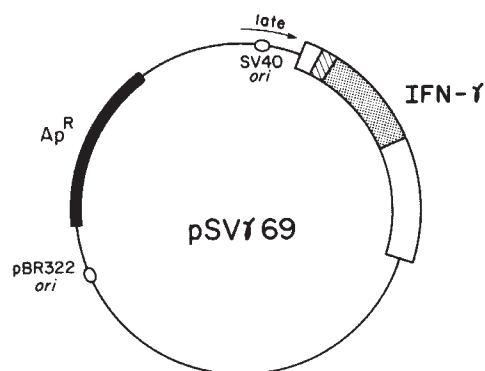
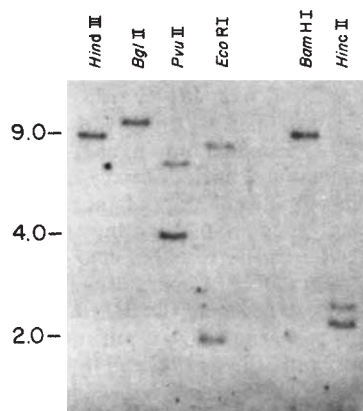


Fig. 5 Plasmid constructed for the expression of IFN- γ in monkey cells. The 342 bp *Hind*III-*Pvu*II fragment encompassing the SV40 replication origin⁶⁰ was converted to a fragment bounded by *Eco*RI restriction sites. The *Hind*III site was converted to an *Eco*RI site by addition of a synthetic oligomer (5'-dAGCTGAATTC) and the *Pvu*II site was converted by ligation to an *Eco*RI site which had been filled-in using DNA polymerase I (Klenow fragment). The *Eco*RI fragment containing the SV40 origin was ligated into the *Eco*RI site of pML-1⁵⁹. The resulting expression vector was chosen which contained the SV40 late promoter oriented to read away from the ampicillin-resistance gene of pML-1. The *Eco*RI site nearest the ampicillin-resistance gene was destroyed by partial *Eco*RI digestion, filling in with the Klenow fragment of DNA polymerase I, and subsequent ligation (as outlined in ref. 73). The 1,250 bp *Pst*I insert of p69 was converted to a fragment bounded by *Eco*RI sites by insertion in the *Pst*I site of pNCV¹³, as the *Pst*I site of pNCV is flanked by *Eco*RI sites. The *Eco*RI-flanked cDNA insert was ligated into the single *Eco*RI site of the pML-SV40 expression vector to give the plasmid shown.

The procedure followed to express the cDNA insert of p69 directly in *E. coli* was similar to that used previously for human growth hormone³⁹, IFN- β (ref. 10) and several IFN- α s^{13,16}, and is outlined in Fig. 4. A *Bst*NI restriction site located at codon 4 of the presumed mature coding sequence was used to remove the signal peptide coding region. Two synthetic deoxyoligonucleotides were designed which restore the codons for amino acids 1-4, incorporate an ATG translational initiation codon and create an *Eco*RI cohesive terminus. These two oligomers were ligated to the remainder of the cDNA insert to construct a 1,115 bp synthetic-natural hybrid gene coding for a polypeptide of 147 amino acids and bounded by *Eco*RI and *Pst*I sites. This gene was inserted into the plasmid pLeIFAtmp103 (a derivative of pLeIFA25 (ref. 13) in which the *Eco*RI site distal to the LeIF A gene was destroyed) between the *Eco*RI and *Pst*I sites to give the expression plasmid pIFN- γ trp48. In this plasmid the cloned gene is transcribed under the control of a 300 bp fragment of the *E. coli trp* operon¹³. This fragment contains the *trp* promoter, operator and the Shine-Dalgarno sequence of the *trp* leader peptide⁵⁸, but lacks the ATG sequence for initiation of translation of the leader peptide. *E. coli* W3110 strains transformed with either pIFN- γ trp48 or p69 were grown and extracted for interferon assay (see Table 1). Extracts from cultures of W3110/pIFN- γ trp48 exhibited definite interferon activity (~250 units per ml of extract), whereas no activity (<3 units per ml) was detected in extracts of W3110/p69.

To express the putative IFN- γ coding sequence of p69 in mammalian cell culture, the expression vector pSV γ 69 (Fig. 5)

Fig. 6 Hybridization of human genomic DNA with a 32 P-labelled cDNA probe from plasmid p69. High molecular weight human lymphocyte DNA (5 μ g) prepared by the procedure of Blin and Stafford⁷⁴ was digested to completion with various restriction endonucleases, electrophoresed on 1.0% agarose gels and blotted to a nitrocellulose filter⁶⁷. The 32 P-labelled DNA probe was prepared from a 594 bp *DdeI* fragment (nucleotides 266–860 of Fig. 3b) of the cDNA insert of p69 as described previously⁴⁵. 1×10^7 c.p.m. of the probe were hybridized with the filter for 16 h and then washed as described elsewhere¹⁴. Two hybridizing DNA fragments were observed with three endonuclease digests: *PvuII* (6.7 and 4.0 kbp), *EcoRI* (8.8 and 2.0 kbp) and *HincII* (2.5 and 2.2 kbp). Three endonuclease digestion patterns provide only one hybridizing DNA fragment: *HindIII* (9.0 kbp), *BglII* (11.5 kbp) and *BamHI* (9.5 kbp).



was constructed from the following three components: (1) the plasmid pML-1 (pML-1 is a derivative of pBR322 in which sequences known to inhibit the replication of SV40–pBR322 recombinants in simian cells have been deleted, but which still contains the ampicillin-resistance selectable marker and an *E. coli* replication origin⁵⁹); (2) a 342 bp *PvuII*–*HindIII* fragment of SV40 virus providing the replication origin of SV40⁶⁰ and which is thought to encompass the promoter for both the early and late SV40 transcriptional units⁶¹; and (3) the entire cDNA insert from plasmid p69.

The cDNA insert and the SV40 origin region were oriented in pSV γ 69 such that the interferon structural gene should be transcribed from the promoter for the late transcriptional unit (Fig. 5). This vector was introduced by DNA transfection into the transformed monkey cell line COS-7 (ref. 62). COS-7 endogenously expresses SV40 large T antigen and has been shown to permit the propagation of recombinant plasmids containing pML-1 and SV40 origin sequences^{59,60}. Cell media from transfected cultures were assayed daily for the presence of interferon activity. Yields of 50–100 units per ml were obtained 3 or 4 days after transfection, indicating that the interferon was being secreted into the culture medium by the COS-7 cells. No interferon activity was observed in the media from cells transfected with plasmids in which the cDNA coding region was inserted in the opposite orientation to that shown in Fig. 5 (data not shown).

Characterization of interferon activity produced by *E. coli* and monkey cells

The properties of the interferon activity present in extracts of *E. coli* W3110/pIFN- γ trp48 and secreted from COS-7/pSV γ 69 cells were compared with authentic human IFN- γ produced by stimulated PBLs and with authentic IFN- α and IFN- β . Distinguishing characteristics of IFN- γ include pH 2 lability and lack of neutralization by antisera prepared against human IFN- α or IFN- β (ref. 2). As indicated in Table 1, the interferon produced by *E. coli* and monkey cells, like the IFN- γ produced by SEB/desacetylthymosin α_1 -stimulated PBLs, was not neutralized by antisera to either IFN- α or IFN- β , although these antisera were effective in neutralizing authentic IFN- α or IFN- β . Antisera prepared against partially purified PBL-produced IFN- γ (E. Rinderknecht and V. Anicetti, unpublished results) completely neutralized the interferon activity synthesized by *E. coli* and monkey cells while having no effect on the activity of IFN- α and IFN- β (Table 1). Furthermore, the complete loss of activity observed when these interferon preparations were treated with pH 2 or 0.1% SDS is characteristic of IFN- γ , and distinct from IFN- α and IFN- β . Therefore, the polypeptide encoded by the cloned cDNA sequence of p69 has the same immunological and chemical properties as authentic IFN- γ .

A single gene for IFN- γ

The human interferon gene family comprises 12 or more distinct IFN- α genes having 80–95% DNA sequence homology^{11–16,63} and at least one IFN- β gene (IFN- β_1 ; refs 64–66) which is 40–50% homologous with the IFN- α genes. To estimate the number of related IFN- γ genes in the human genome, human DNA of one individual was digested with several restriction enzymes, electrophoresed through agarose gels, transferred to nitrocellulose paper⁶⁷ and hybridized to a 32 P-labelled IFN- γ cDNA probe (Fig. 6). *HincII* is the only one of the six restriction enzymes used that cleaves the IFN- γ cDNA sequence, but the *HincII* site is not located in the DNA fragment which was used as probe. A unique, intronless gene would thus have appeared as a single hybridizing DNA fragment in each lane of the gel. Instead, two hybridizing DNA fragments were observed for three of the endonuclease digestions (*PvuII*, *EcoRI*, *HincII*) and single hybridizing fragments of about 9 kilobase pairs (kbp) each were found for the other digestions (*HindIII*, *BglII*, *BamHI*). Similar genomic blots using DNA from other individuals gave the same hybridization patterns. There are two possible explanations for these results: first, there are two homologous and closely linked (<8 kbp apart) IFN- γ genes separated by *PvuII*, *EcoRI* and *HincII* sites, and second, there is a single IFN- γ gene which contains one or more introns containing *EcoRI*, *PvuII* and *HincII* sites. This second possibility was supported by repeating the hybridizations with a 32 P-labelled probe prepared from the 3'-untranslated region of IFN- γ cDNA. In this experiment only single *PvuII*, *EcoRI* and *HincII* fragments hybridized to the probe (data not shown). The gene structure of IFN- γ is therefore distinctly different from that of IFN- α and IFN- β , which contain no introns^{15,63–66}.

Discussion

Poly(A) mRNA from human lymphocytes producing IFN- γ was fractionated on a denaturing agarose gel. The 18S size fraction was used to prepare a cDNA library in *E. coli*. Twenty-two bacterial clones containing potential IFN- γ sequences were identified by specific hybridization to 32 P-cDNA probes prepared using mRNA from induced lymphocytes and failure to hybridize to similar probes prepared from uninduced mRNA. Restriction mapping and DNA hybridization experiments revealed that all 22 induced cDNA clones were related and that the frequency of these clones in the cDNA library was about 1:120. Therefore, the frequency of IFN- γ mRNA in our most active poly(A) RNA preparation is $\sim 1:2,400$ because the mRNA used to prepare this cDNA library had been enriched about 20-fold in IFN- γ sequence by the agarose gel fractionation procedure.

The 1,194 bp cDNA insert (excluding poly(A) sequence and dG·dC tails) of p69 codes for a polypeptide of 166 amino acids, 20 of which are probably cleaved during the process of secretion of mature IFN- γ from lymphocytes. The encoded amino acid sequence, which bears little resemblance to those of IFN- α or IFN- β , has two potential glycosylation sites and is quite basic in character. Identification of this sequence as IFN- γ was obtained by expression of the cDNA sequence of p69 in bacterial and mammalian cells. In both systems the expression product was shown to have antiviral activity which was immunologically indistinguishable from that of the IFN- γ produced by stimulated human lymphocytes. Additionally, the SDS and pH 2 sensitivity observed for the IFN- γ produced by bacteria and monkey cells are distinguishing features of authentic IFN- γ (refs 2, 18–21).

The amounts of antiviral activity recovered from *E. coli* producing IFN- γ are significantly lower than those obtained for IFN- α or IFN- β using similar expression vectors^{10,13,16}. If a specific activity range of 10^8 – 10^9 units per mg is assumed for IFN- γ (ref. 20), between 8 and 80 active molecules are present per cell. However, if the specific activity of the interferon depends to some degree on its state of glycosylation, considerably more interferon molecules could be present per cell. Additionally, if we were incorrect in our assumptions about which

amino acids constitute the signal peptide, the molecule expressed in *E. coli* may not exhibit the same specific activity as natural IFN- γ . If one assumes that authentic mature IFN- γ is a 146-amino acid polypeptide, its molecular weight, minus carbohydrate, would be 17,110. The difference between this value and the published molecular weights of 35,000–70,000 for IFN- γ probably cannot be accounted for by glycosylation alone. It is conceivable that authentic IFN- γ is associated with carrier proteins in serum or exists as a multimer of identical subunits. The purification and sizing of IFN- γ synthesized by *E. coli* and monkey cells should help resolve this discrepancy in molecular weight.

The human IFN- γ gene corresponding to the cDNA sequence described here seems to contain at least one intron and to have

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LETTERS TO NATURE

Is the remnant of SN1006 composed of iron?

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The remnant of the supernova explosion of AD 1006 (the brightest in recorded history) has a shell-like appearance at both radio and X-ray wavelengths. Einstein X-ray observations by Pye *et al.*¹ show a soft (photon energy $\epsilon \leq \frac{1}{2}$ keV) filled interior surrounded by a harder ($\epsilon \geq 1$ keV) unresolved ring of emission of radius ~ 15 arc min. Superficially it appears to be similar to most supernova remnants for which many studies have shown that the X-ray emission is thermal radiation from gas shock-heated by high-velocity ejecta. The spectrum² of parts of the

no additional genomic homologues. It remains to be determined, however, whether additional nonhomologous genes exist which code for proteins meeting the criteria required for classification as IFN- γ (refs 1, 2).

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edge of the remnant in the range $0.75 \leq \epsilon \leq 10$ keV shows, however, no evidence for line emission. The spectral shape is consistent with a power-law perhaps due to a non-thermal mechanism. Moreover, the total mass of gas¹ required exceeds $\sim 10 M_{\odot}$ if it is of normal cosmic abundance. This is much greater than that expected for a remnant several hundred parsecs above the galactic plane. As no pulsar (or Crab-like morphology) is evident in the remnant of SN1006, Reynolds and Chevalier³ have proposed that relativistic particles are being efficiently accelerated in shocked gas, and provide the major source of X rays through synchrotron emission. SN1006 is then an important region for the study of such acceleration processes. Here we reinvestigate a thermal interpretation and show that the element abundances associated with a Type I supernova^{4–7} may provide an alternative explanation.

First we note that the observed lack of line emission from the edge of the remnant requires that sulphur, silicon and iron be at less than half their cosmic value relative to hydrogen². We suggest that SN1006 was a Type I supernova of a few solar masses exploding into its own stellar wind (outer density $\rho \propto$

r^{-2}). If the progenitor star was a pop II object (appropriate for its galactic position) then it is quite reasonable to suppose that the metal abundances are lower than the cosmic values. It is also possible that most of the metals are in the form of grains, which have not yet had time to combine with the hot gas. The stellar wind may have been associated with a red-giant phase (compare with the formation of planetary nebulae) or mass-transfer from a binary companion. The unresolved outer ring of emission¹ is then due to the shocked wind material. Gas shocked earlier (and closer to the contact discontinuity) is now denser and cooler than gas that is now just being shocked and swept up. Bremsstrahlung from a range of densities and temperatures in the shocked wind can then mimic a power-law spectrum.

The mass of the interior region has been estimated¹ as $\sim 6M_{\odot}$, if due to a gas of cosmic abundance (the temperature is $\sim 10^6$ K). Most of the emission is strongly peaked below 0.25 keV and could be completely due to lines. (The spectral resolution of the detector used for these observations is $\sim 100\%$ at these energies.) It has been widely suggested⁴⁻⁷ that $\sim 1M_{\odot}$ of ^{56}Fe is ejected in a Type I supernova explosion. Line emission from such material can easily provide the interior X-ray flux from SN1006 if it has a distance of ~ 1 kpc. Interstellar absorption corresponding to a column density of $N_{\text{H}} \approx 5 \times 10^{20} \text{ cm}^{-2}$ is required to make this hypothesis consistent. One clear prediction is the possibility of detecting coronal line emission from the interior of the remnant in the optical. We expect a flux of $\approx 2 \times 10^{-7} \text{ erg s}^{-1} \text{ cm}^{-2} \text{ sr}^{-1}$.

The overall 0.2–2.5 keV spectrum⁸ of SN1006, after subtraction of a contribution due to the Lupus Loop, does show evidence of an emission feature at ~ 0.6 keV. This is identified in ref. 8 as oxygen line emission, but we note that line emission due to calcium, which is also likely to be produced in a Type I outburst, occurs at a similar energy.

One major problem with any thermal interpretation of the X-ray observations lies in maintaining the interior of the remnant at 10^6 K. Adiabatic cooling on the expansion time scale erases all memory of the initial phase of expansion of the remnant. All but large scale velocity inhomogeneities should have decayed and the effects of radioactivity will have died away. We propose that a reverse shock⁶ propagated back through the material at an age of a few hundred years. This requires the ejecta to have collided with roughly its own mass in that time. Perhaps the pre-supernova was surrounded by a wind with $\rho \propto r^{-2}$ at large radii ($R \leq 10^{18} \text{ cm}$), but with ρ constant (and total mass $\sim 1 M_{\odot}$) at small radii (the wind velocity may have decreased and M increased) just before the supernova explosion. We have verified that present-day interior temperatures of $\sim 10^6$ K and reasonable luminosities are obtainable from such a situation by numerical computation of a spherically-symmetric explosion. The remnant is now freely expanding. No definite conclusion may be drawn, although the structure of young remnants depends strongly on the initial conditions in the ejecta and surrounding material. We consider that a complex structure is quite likely and that the appearance of young supernova remnants may be quite variable. The unusual properties of SN1006 discussed here may be partly due to its galactic location. The low temperature interior can be easily detected as the line-of-sight column density is low, and the expected low density of the surrounding interstellar medium allows a stellar wind to propagate freely to relatively large radii.

Optical confirmation that the interior of SN1006 is mostly composed of iron would strongly support the ^{56}Ni – ^{56}Co – ^{56}Fe radioactive interpretation of Type I light curves⁴⁻⁷ and the related theoretical interpretation of the explosion itself. It would also make a more detailed discussion of the above admittedly *ad hoc* picture worthwhile. As discussed earlier, any more-normal abundance of a thermal gas requires that the progenitor was $>10 M_{\odot}$, although it is out of the galactic plane by several hundred pc. This seems most unlikely and suggests that failure to detect coronal lines will provide support for rapid and efficient acceleration of cosmic rays in shocks (see ref. 3).

We conclude by considering the cup-shape appearance of the remnant of SN1006, as it appears in the X ray¹. This could partly be due to the exploding star having been a member of a close binary system. The flow and subsequent shocks surrounding the companion could well have slowed the ejecta down in one direction, provided that the ejecta was roughly at its final velocity by the time it had crossed the binary orbit (say $\sim 10^{11} \text{ cm}$).

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Asymmetric Balmer line profiles in Seyfert galaxies

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The profiles of the broad Balmer emission lines in Seyfert galaxies are often asymmetrical in the sense that the red wing extends further than the blue wing¹. Explanations of the observed asymmetry have involved relativistic effects^{2,3}, infalling clouds immersed in an absorbing medium⁴, and radially expanding clouds that are bright only on the side facing the ionizing source^{4,5}. In all these hypotheses, the fact that only some galaxies have asymmetric emission lines was not considered important. Here I present evidence that both the degree of asymmetry and the difference between $H\alpha$ and $H\beta$ profiles are correlated with the overall line width. I also argue that current evidence is most compatible with an expanding system of optically thick clouds, in which the thickest clouds are moving fastest.

In his study of 36 Seyfert galaxies, Osterbrock¹ classified each $H\beta$ profile as either symmetric, slightly asymmetric, or asymmetric. Figure 1 shows that all the 'asymmetric' objects have large values of the full width at zero intensity (FWZI) of $H\alpha$. There is a large overlap between the 'symmetric' and 'slightly asymmetric' populations, but in a probabilistic sense the 'slightly asymmetric' objects have values of FWZI ($H\alpha$) intermediate between the 'symmetric' and the 'asymmetric objects'. The profiles were not corrected for the instrumental resolution of the Lick scanner. As the instrumental profile¹ has FWZI $\sim 2,000 \text{ km s}^{-1}$ at $H\beta$, it is clear that the narrowest lines would always appear symmetric. However, most of the lines concerned are well resolved, and some of the observed asymmetries are very strong; the resolution effect cannot explain the proposed correlation.

Osterbrock¹ noted that an apparent asymmetry may actually be due to the broad weak feature Fe II $\lambda 4,924$ appearing in the red wing of $H\beta$. I considered the strength of the well observed feature Fe II $\lambda 4,570$, assuming it to correlate with Fe II $\lambda 4,924$. There is no significant difference between the three populations (symmetric, slightly asymmetric, and asymmetric) for their distributions of the value of Fe II $\lambda 4,570/H\beta$ (not shown). Thus, an apparent asymmetry is probably not caused by Fe II emission dominating the wings of $H\beta$. Of course, some extra, as yet undetected, component could appear redward of $H\beta$, and thus

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also increase the apparent overall line width. The FWZI is very difficult to measure, so that there may be important unknown systematic errors. However, I shall assume that the asymmetry, and its dependence on line width, is a real feature of the Balmer line profiles, and attempt to explain it in terms of the properties of the emission line cloud system as a whole. Let us reconsider the three categories of explanation mentioned above in the context of a dependency on line width.

(1) Objects with extremely broad lines must contain emission line gas moving at mildly relativistic velocities. Furthermore, if the clouds are bound to a massive central object and have virialized velocities, those moving fastest also have the largest gravitational redshift, and transverse Doppler shift (also always a redshift). We need not assume, as did Netzer² that the clouds are rotating in a disk, but only that typical velocities at each radius are comparable with the orbital velocity at that radius. These assumptions do predict that excess redward emission is increasingly important for objects with very fast moving clouds. Unfortunately, however, the effect is probably too small to explain at least some asymmetries. For clouds orbiting a massive body at $\sim 15,000 \text{ km s}^{-1}$, we would expect that the red wing may extend $\sim 10\%$ further than the blue wing, whereas in some cases the red wing actually extends twice as far as the blue wing (see, for example, the profile of MKN374 reproduced in Fig. 1 of ref. 4, or the profile of PHL1093 reproduced in Fig. 1 of ref. 3). For instance, the profile of PHL1093 extends to $-5,000 \text{ km s}^{-1}$ and $+10,000 \text{ km s}^{-1}$. This would require that clouds exist within 30 Schwarzschild radii of the massive central object, but move slower than the corresponding orbital velocity by a factor of 7.

(2) A consensus is forming that the anomalous ratios of $\text{Ly}\alpha/\text{H}\alpha/\text{H}\beta$ observed in QSOs and Seyfert galaxies imply that the broad line emitting clouds are optically thick to the Balmer lines⁵⁻¹². This means that the inner, ionized face of the cloud (that closest to the nucleus) will be brighter in the Balmer lines than the outer face. Most Balmer line photons detected by the observer will thus have originated in clouds on the far side of the ionizing source. A red excess then implies that the clouds on the far side are moving away from us; that is, the system is expanding radially outward^{4,5}. The correlation found here between the degree of asymmetry and FWZI ($\text{H}\alpha$) (Fig. 1), further requires that faster moving clouds have a larger contrast in brightness between inner and outer faces, and thus presumably a larger Balmer line optical thickness (see Fig. 1 of ref. 5).

(3) Alternatively, one may consider isotropically emitting clouds immersed in an obscuring medium (for example, dust). Clouds on the far side of the system are seen through a larger column of the obscuring material, and are thus fainter. A fainter blue wing then means that clouds on the far side are moving towards us; that is the system is moving radially inward. The correlation between asymmetry and line width would then imply that objects with very wide lines have a larger overall amount of

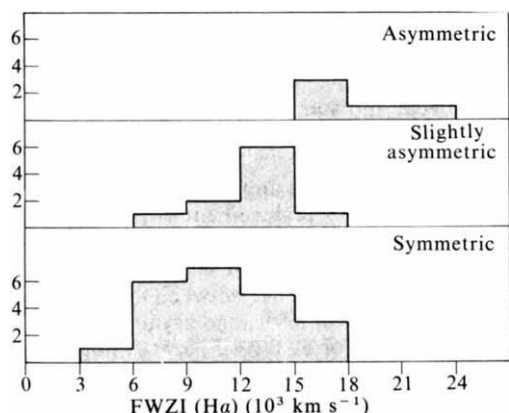


Fig. 1 Distribution of the values of the full width at zero intensity of $\text{H}\alpha$ for the three different profile symmetry classes defined by Osterbrock¹.

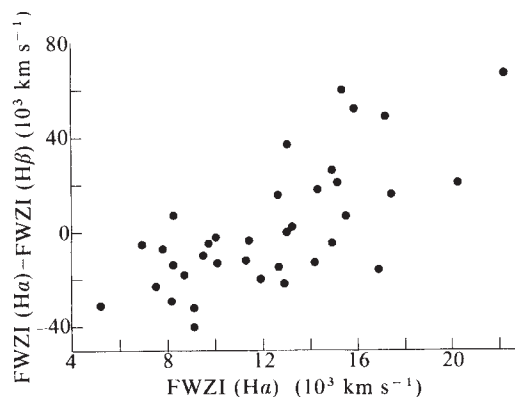


Fig. 2 The difference in full width at zero intensity between $\text{H}\alpha$ and $\text{H}\beta$ as a function of the full width at zero intensity of $\text{H}\alpha$. Values are from ref. 1.

obscuring material. However, where those 'asymmetric' galaxies (all except MKN374) have been measured in X rays, they are all as luminous as, or more luminous than, the average Type 1 Seyfert galaxy^{13,14}. By contrast, much evidence now suggests that only low luminosity Seyfert galaxies have appreciable amounts of obscuring material¹⁵.

There is further evidence that supports model (2). Figure 2 shows that the line-width difference, $\text{FWZI}(\text{H}\alpha) - \text{FWZI}(\text{H}\beta)$, is an increasing function of $\text{FWZI}(\text{H}\alpha)$. Such a correlation must be treated cautiously, as FWZI is difficult to measure. The value of FWHM is less ambiguous, but will be dominated by the relative strength of the narrow component of the emission line, which will have little bearing on the dynamics of the region forming the broad lines.

Line profiles have been described as 'logarithmic'⁴ or 'triangular'¹⁶. In the latter case, FWZI is a better defined quantity than in the former case; but in general one may suspect that the measured value of FWZI is as much a function of the signal-to-noise ratio as intrinsic line width. In this situation FWZI could correlate with line strength; a strong line may be traced out further from the line centre. The difference in FWZI between $\text{H}\alpha$ and $\text{H}\beta$ could then correlate with the ratio of line strengths. However, from Osterbrock's data, there is no correlation between $I(\text{H}\alpha)/I(\text{H}\beta)$ and $\text{FWZI}(\text{H}\alpha) - \text{FWZI}(\text{H}\beta)$ (not shown). Such signal-to-noise effects may still cause a correlation of line width difference with overall line width if, regardless of the total line intensities, $\text{H}\alpha$ 'fades out' more slowly far from the line centre than does $\text{H}\beta$; that is to say, if the Balmer decrement is a function of cloud velocity. This suggests that, in general, different objects all have the same 'potential' profile; it is just a question of how much of the 'iceberg' is above water.

A consistent physical picture is that clouds are moving outward; that faster moving clouds are optically thicker; and that therefore they have a larger brightness contrast between inner and outer faces, and larger values of $\text{H}\alpha/\text{H}\beta$. Examination of these questions at higher resolution and sensitivity is desirable, as is examination of other spectral lines.

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A molecular interpretation of stress corrosion in silica

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The mechanical strength of many glasses and ceramic materials decreases with time under static loading and ambient environments. This strength loss is associated with slow growth of pre-existing surface flaws due to a stress-corrosion process. To make long-term strength predictions for ceramic components, it is important to understand the stress-corrosion mechanism. We have studied stress corrosion in vitreous silica exposed to water and several non-aqueous environments and report here that environments which enhance stress corrosion are composed of molecular groups with electron donor sites on one end and proton donor sites at the other. These results suggest a detailed mechanical model for the interaction of the environment with mechanically strained bonds in the solid at the tip of a crack. The proposed model also has implications for the long-term strength behaviour of a wide variety of brittle materials.

Several generalized mechanisms have been proposed to explain the effect of water-containing environments on crack growth in glass, for example, surface energy reduction¹, stress-enhanced dissolution², or dielectric charge compensation³. None of these models attempts to explain why water is such an effective stress-corrosion agent for glasses and ceramics nor does any suggest a specific stress-enhanced reaction. We now describe, on a molecular scale, a specific chemical reaction between strained bonds in vitreous silica and water, which can be used to explain environmentally enhanced crack growth. Experimental support of this model will be presented.

Previously published results⁴ have shown that slow extension ($<10^{-2} \text{ m s}^{-1}$) of a crack in vitreous silica is facilitated by water in the environment. In the present study, crack growth data were taken in N_2 gas containing a small amount of water as well as in liquid water and other environments. Crack velocities were obtained by visual observation of crack extension in a double cantilever beam specimen⁵, and the data plotted as crack velocity (V) versus stress intensity factor (K_I), where K_I is a measure of the stress field at the crack tip (Fig. 1). The important features are:

(1) In a water-free environment such as vacuum, spontaneous failure is observed when K_I reaches a critical value⁴ (shown as a vertical line in Fig. 1).

(2) When small amounts of water vapour are present (moist N_2 gas), crack growth in the low velocity regime is enhanced (curve is shifted to lower K_I and V is a measurable function of K_I).

(3) A plateau in crack velocity is observed in water-containing environments (such as moist N_2). This plateau has been linked to crack growth limited by the rate of water transport to the tip of the moving crack⁶. At sufficiently high V , the plateau behaviour ends and rapid fracture is observed.

(4) Liquid water enhances crack growth over the entire range of crack velocity (10^{-9} – 10^{-2} m s^{-1}).

Highly concentrated tensile stress fields are produced at a crack tip in a stressed brittle solid. Continuum approximations indicate that the bridging Si—O bond experiences strains $>20\%$. The effect of this strain on the bonding molecular orbitals is not easy to predict but can be discussed in terms of a decrease in the overlap between atomic orbitals, thus increasing their availability for bonding with other species.

The molecular structure of water is the key to its ability to cause stress corrosion in vitreous silica. Oxygen atomic orbitals

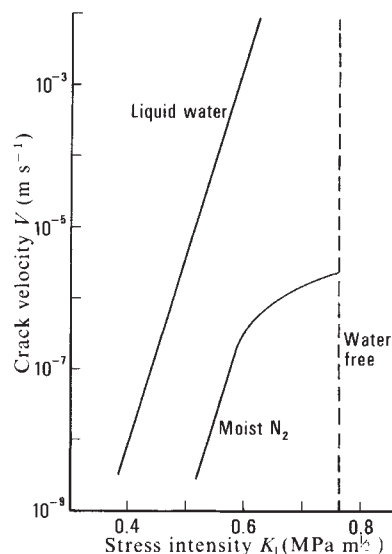


Fig. 1 Effect of water on slow crack growth in vitreous silica (curves represent best fit to experimental data).

2s, 2p_x, and 2p_y form three hybrid orbitals—two of which σ -bond with hydrogen atoms. The remaining lone pair orbitals (one hybrid and the remaining p_z) are directed away from the hydrogen atoms. This arrangement results in a positive charge centre at the hydrogen end of the water molecule and a negative charge centre on the opposite end of the molecule where the lone pair orbitals are located.

Interaction between strained bridging bonds at a crack tip in silica and water from the environment can be represented by a three-step process (see Fig. 2).

Step 1—a water molecule from the environment attaches to a bridging Si—O—Si bond at the crack tip. The water molecule is aligned by: (1) formation of a hydrogen bond with the O_{br} atom; and (2) interaction of the lone pair orbitals from O_w with the Si atom. The lone pair orbital interaction may involve either Van der Waals attraction or some covalent bonding with unoccupied d orbitals of Si.

Step 2—a concerted reaction occurs in which proton transfer to the O_{br} is accomplished simultaneously with electron transfer from the O_w to the Si atom. As a result of this reaction, two new bonds are formed (one between O_w and Si and one between hydrogen and O_{br}; the original bridging bond (between O_{br} and Si) is destroyed.

Step 3—rupture of the hydrogen bond between O_w and transferred hydrogen occurs to yield surface Si—O—H groups on each fracture surface. As the hydrogen bond is weak, this step is expected to occur immediately after proton transfer. (Budd⁷ has proposed a similar mechanism for the dissolution of silica in water; however, note that the present model for crack growth is not contingent on the removal of material from the fracture surface.)

Reaction rate theory⁸ can be used to show how the proposed mechanism for bond rupture leads to enhanced crack growth rates. In vacuum, the entire energy of the bridging bond must be overcome before bond rupture can occur. (This energy is supplied in part by mechanical strain as well as by thermal contributions.) When water is present, the height of the energy barrier to bond rupture is reduced because charge transfer (step 2) provides an alternative, lower energy, reaction path. As the rate of a chemical reaction (in this case bond rupture) is reciprocally dependent on the exponent of the energy barrier, mechanisms that provide a lower energy activated state will enhance crack growth rates. We have omitted factors resulting from changes in the energy of the Si—O—Si bond due to adsorbed water (step 1) and the energy of the reaction products (step 3); these terms are not critical to this discussion.

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Experimentally, the role of the concerted reaction (simultaneous electron and proton transfer) as the rate of limiting step to fracture can be borne out through isotopic effects. Results of fracture experiments performed in deuterated water produced crack velocities consistently lower than those attained in normal water. This result can be analysed in terms of the different ground state vibrational energies of deuterium and protium resulting in different activation barriers for the two isotopes. The lower crack velocities measured in deuterated water agree with the larger energy barrier expected for deuterium transfer⁹.

This model for chemical bond rupture presents some interesting implications. First, it suggests that environments other than water should enhance cracking in silica glass, if the species possess structural and bonding features similar to water, that is proton donor sites at one end of the molecule (or group) and lone pair orbitals at the other. The environmental molecule must also fit between the Si—O bond ($\approx 1.63 \text{ \AA}$). To test this hypothesis, crack growth studies were conducted in vitreous silica exposed to gaseous ammonia (NH_3). Ammonia has a structure similar to water except that it has three orbitals σ -bonded to hydrogens leaving one lone pair orbital directed away from the hydrogen atoms (the N—H bond length is $0.94 \text{ \AA}$). Figure 3 shows that NH_3 enhanced crack growth over the entire velocity range. When evaluating such data, it is important to be aware that small amounts of water in an inert carrier (N_2) can have large effects on crack growth rates. However, the enhancement due to small amounts of water is limited in velocity. As the NH_3 gas used in this experiment contained less H_2O than the $\text{N}_2 + \text{H}_2\text{O}$ gas mixture, the absence of a plateau indicates that the NH_3 was controlling the fracture behaviour. Crack growth experiments in hydrazine (N_2H_4) and formamide (CH_3NO) showed that these environments also controlled crack growth over the entire velocity range (Fig. 3). However, these environments were less effective (V - K_I curve shifted to higher K_I) than either water or ammonia, possibly due to steric hindrance and/or delocalization of lone pair orbitals. Note also that other non-aqueous environments such as carbon monoxide (contains lone pair orbital but no proton donor site) or acetonitrile (has high dipole moment, 3.92 D , but no localized lone pair orbital) have no measurable effect on crack growth rates.

This chemical model and its supporting experimental data represent an important step in the understanding of stress corrosion and the mechanical durability of materials. First, knowing that an effective stress-corrosion environment must have an electron donor site as well as a proton donor site allows for qualitative predictions of those environments which will decrease the long term strength of silica. As the structural bonds in the solid must also engage in the stress-corrosion reaction, qualitative predictions of the susceptibility of a range of solids to stress corrosion are also possible, that is the more covalent the solid the less susceptible it is to stress corrosion. This is supported by experimental evidence that single crystal silicon shows little, if any, susceptibility to stress corrosion with water (E. R.

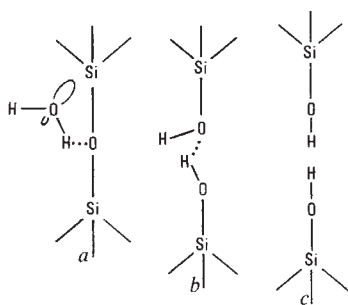


Fig. 2 Representation of the proposed reaction between water and a strained Si—O—Si bond at the crack tip. Reaction steps involve; (a) adsorption of water to Si—O bond, (b) concerted reaction involving simultaneous proton and electron transfer, and (c) formation of surface hydroxyls.

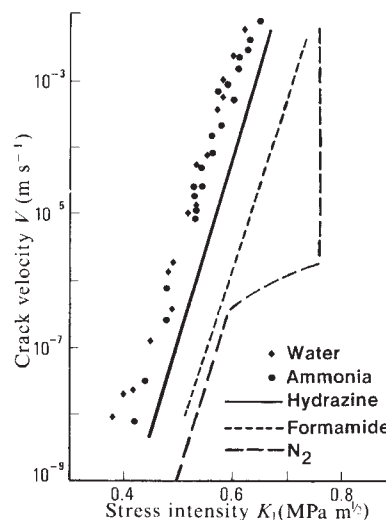


Fig. 3 Comparison of the effects of various environments on slow crack growth in vitreous silica at room temperature.

Fuller, personal communication). The ability to make such qualitative predictions is an advance over previous models which were intended only to explain stress corrosion in water.

Second, this attempt at analysing the molecular interaction involved in the stress-corrosion process opens the door for detailed molecular calculations. Results of such calculations could allow quantitative predictions of the response of any solid material with any particular stress-corrosion environment.

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Errors and resolution of thermal techniques for obtaining the geomagnetic intensity

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Techniques designed to obtain the intensity of the geomagnetic field by thermal demagnetization are subject to error due to differences between the ancient and laboratory cooling rate^{1–3} and alteration in the physical and chemical properties of the magnetic grains which can lead to a systematic overestimate of the field by as much as 50%. A new technique is proposed here which uses the change in moment with time at a fixed temperature in conjunction with the magnetization acquired on cooling to determine the ancient field and ancient cooling rate. This new technique also appears to be able to detect alteration with greater sensitivity.

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It has been found that the magnitude of both the above errors increases with temperature. Alteration often begins at relatively low temperatures, and increases smoothly as the temperature increases. The cooling rate correction in effect also increases with temperature. If alteration results in a decrease in magnetization the result is an accelerating decrease in moment with increasing temperature. Consequently the abrupt change in the slope of a natural remanent magnetism (NRM)/palaeothermal remanent magnetism (PTRM) plot which has been used to signal the onset of alteration is not always present. This will result in an accelerating apparent increase in the intensity of the ancient field. An extreme example of this behaviour is shown in Fig. 1: while the NRM/PTRM plot settles down to a straight line above 300 °C the grains involved have probably undergone alteration. Thus the reliability of using the slope of the straight line portion is questionable.

The technique described here not only corrects for the difference between laboratory and ancient cooling rates, but enables the ancient cooling rate to be estimated. Preliminary results indicate that the ancient cooling rate could vary by more than an order of magnitude. Thus the error due to differences in cooling rate can be random even for pottery. These results will also reveal that alteration begins to appear at surprisingly low temperatures.

As derived in ref. 1, the moment produced on cooling is

$$M = H_A \int_{v_1}^{v_2} \frac{J_B J}{3kT_B} v^2 N(v) dv \quad (1)$$

where H_A is the ancient field, J_B the saturation magnetization at the blocking temperature T_B , v is the grain volume, $N(v)$ the grain size distribution and v_2 and v_1 the volumes of grains blocked at the higher temperature and lower temperature respectively.

The quantities J_B and T_B depend on the rate of cooling¹. Let a

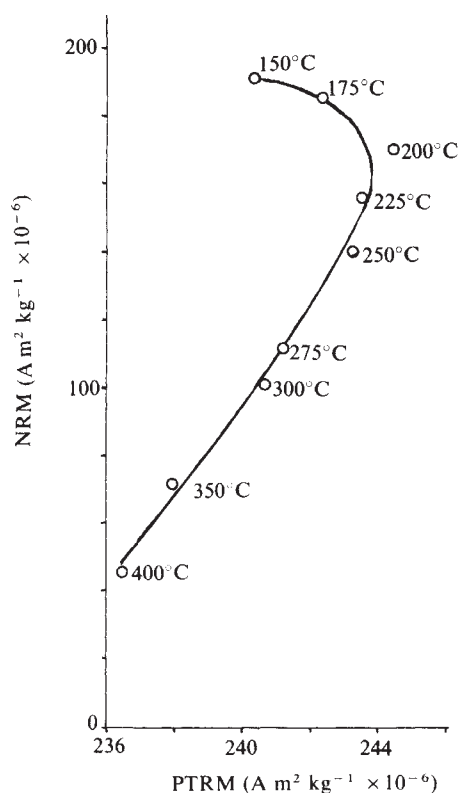


Fig. 1 The change in moment of a sample of Attic pottery after successive heatings to the temperatures (○). Two coolings are performed from each temperature, the first in a field of 70 μT, the second in zero field to establish the loss of the NRM.

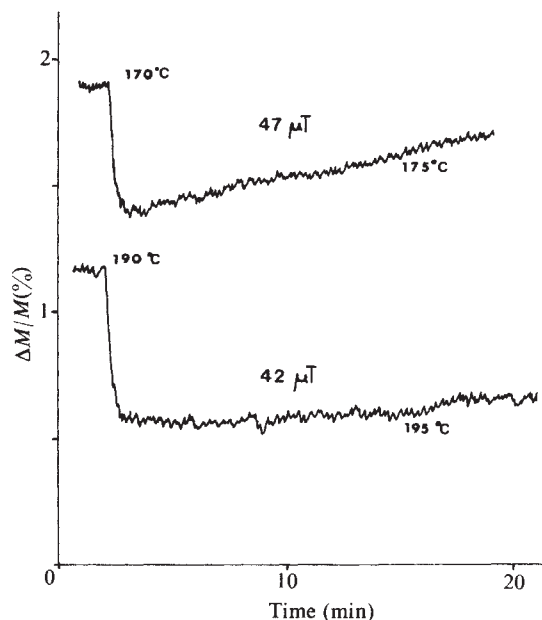


Fig. 2 The change in moment, M , with time of a sample of Attic pottery on heating by 5 °C from two temperatures at two different fields. The zero level for both curves has been shifted by an arbitrary amount.

variable α be defined as

$$\alpha = \frac{d}{dt} \left(\frac{K}{T} \right) \quad (2)$$

where K is the anisotropy constant.

The moment is then proportional to $(\ln ck/v\alpha)^n$ where c is a frequency factor.

A cooling time constant τ can be defined as

$$\tau = \frac{k}{v\alpha} \quad (3)$$

In general τ is temperature dependent. In particular if the sample cools according to Newton's law of cooling, τ decreases as the temperature increases.

The coefficient n results from approximating the temperature dependence of J as

$$J = J_0(1 - T/T_C)^n \quad (4)$$

For magnetite $n \approx \frac{1}{2}$ while for haematite it is $\frac{1}{4}$.

The thermal techniques for obtaining the ancient field pioneered by Thellier, involve comparing a moment introduced on cooling in the laboratory field with that removed on heating to successively higher temperatures. A knowledge of the ancient cooling rate is necessary for an accurate determination of the ancient field intensity. This knowledge need only be approximate because the function $(\ln c\tau)^n$ is only weakly dependent on τ .

Assuming that the sample is heated rapidly from a temperature T_0 to a higher temperature T , when the sample reaches T part of the magnetic moment will reside in grains whose blocking temperatures are both above and below T . Those grains whose blocking temperatures are lowest will first come to equilibrium with the applied magnetic field. But the approach to equilibrium of grains whose blocking temperatures are less than T will result in a loss of moment; this produces a decrease in moment followed by an increase when the distributions of those grains with blocking temperatures higher than T begin to reach equilibrium.

The rate of change of the moment¹ with $\ln ct$ is

$$\frac{\partial M}{\partial \ln ct} = \frac{\pi\sqrt{2}}{e} (n_0 - n_{eq}) J \left(\frac{kT}{K} \right)^2 (\ln ct) \left[N \left(\frac{kT}{K} \ln ct \right) \right] \quad (5)$$

n_0 is determined by the moment distribution produced on cooling and is

$$n_0 = \frac{J_B v H_A}{3kT_B} \quad (6)$$

and

$$n_{eq} = \frac{J^v H_L}{3kT} \quad (7)$$

where H_L is the laboratory field.

As the sample is held at the temperature T , initially smaller grains will come to equilibrium followed by grains of increasingly larger volume. Initially, then, the distribution of the grains with low blocking temperature come to equilibrium followed by grains which blocked at higher temperatures. Since T_B increases and J_B decreases as v increases, n_0 will decrease with time. On the other hand, n_{eq} increases with time as v increases.

Now $\partial M / \partial \ln ct$ will be zero when $n_{eq} = n_0$, when

$$\frac{H_A}{H_L} = \frac{JT_B}{J_B T} \quad (8)$$

Now

$$\frac{\bar{K}_B v}{3kT_B} = \ln c\tau \quad \text{and} \quad \frac{\bar{K} v}{3kT} = \ln ct \quad (9)$$

where t is the time at which $\partial M / \partial \ln ct = 0$ and $\bar{K}_B$ is the anisotropy constant evaluated at the blocking temperature.

So if

$$\bar{K} = aJ^p \quad (10)$$

$$\frac{J}{J_B} = \left(\frac{T \ln ct}{T_B \ln c\tau} \right)^{1/p} \quad (11)$$

Using equation (4)

$$\frac{J_B}{J} = \left(\frac{1 - T_B/T_c}{1 - T/T_c} \right)^n = \left(1 + \frac{T - T_B}{T_c - T} \right)^n$$

Bearing in mind that T_B is close to T this equation may be expanded. After some algebra equation (8) becomes

$$H_A \ln c\tau_A = H_L \ln c\tau \left\{ 1 + \left[\frac{(p-1)n}{(pn-1) + T/T_c} \right] \times [\ln(t/\tau_A) / \ln c\tau_A] \right\} \quad (12)$$

where τ_A is the ancient cooling time constant.

Figure 2 displays the moment as a function of time for a sample of Attic pottery. The data were obtained with a highly sensitive magnetometer capable of obtaining moments, while the sample is held at an elevated temperature, with an accuracy of better than 0.1%. It will be described in detail elsewhere.

Since there are two values for H and two values of t , an approximate value for c may be deduced, if the second term in the brackets in equation (12) is neglected, c is $\sim 7 \times 10^7 \text{ s}^{-1}$.

It is impossible to obtain the ancient field unless τ_A is known, although it need not be known precisely because it enters equation (12) as $\ln c\tau_A$. This requires another relation which can be obtained from the dependence of the moment on the laboratory cooling rate, as the moment gain on cooling is proportional to $(\ln c\tau_L)^n$. For example, on further heating to 250 °C the sample revealed a decrease in moment when cooled to 190 °C relative to that observed after it had been cooled from 195 °C. On decreasing the cooling rate by an order of magnitude (increasing τ_L by a factor of 10) half the lost moment was recovered. Thus a two order of magnitude increase in τ_L would be required to restore completely the moment lost between 195 and 250 °C. As shown in ref. 1 the moment introduced on cooling is proportional to $H_L (\ln c\tau_L)^n$. Hence,

$$H_A (\ln c\tau_A)^n = H_L (\ln c\tau'_L)^n \quad (13)$$

where τ'_L is that cooling time constant that would be required to restore completely the moment lost by heating to 250 °C.

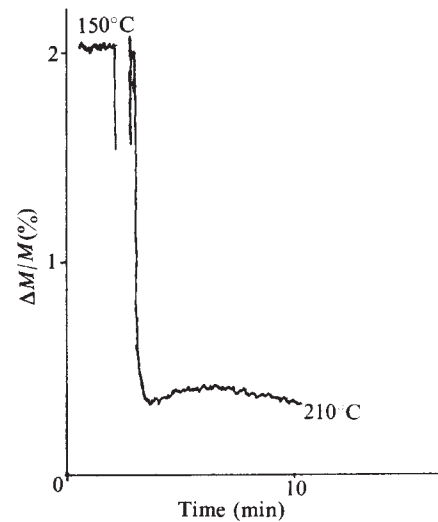


Fig. 3 The change in moment, M , with time of a sample of Attic pottery on heating to 210 from 150 °C. The zero is arbitrary, and two changes of zero suppression were made to keep the recorder pen on scale. The decrease in moment apparent after about 7 min is due to alteration of the magnetic constituents.

Using equation (12), and taking $n = \frac{1}{2}$ which is appropriate for magnetite, $\tau_A \sim 100 \text{ s}$ —a rather low value. Other samples of Attic pottery yielded values about an order of magnitude higher. Typically τ_A appears to be of the order of 1,000 s for this pottery.

H_A for this sample is 46 μT . The modified Thellier method used for a previous determination yielded $H_A = 63 \mu\text{T}$ for a sample from the same sherd. The difference is rather more than can be accounted for by differences in cooling rate. Alteration probably affected the old result.

This technique consists of holding the sample at an elevated temperature and monitoring the change in its moment with time. If the laboratory field is within $\sim 10\%$ of the ancient field the moment will show a characteristic decrease followed by an increase. The time at which the inflection occurs yields the product $H_A \ln c\tau_A$. The values for $\ln c\tau_A$ and H_A can be separated by obtaining the laboratory cooling rate for which no change in moment occurs on cooling to a fixed temperature in the chosen laboratory field.

With this particular material the onset of mineral alteration seems to result in a net loss of moment by the grains. This has manifested itself by an anomalously large τ_L . Thus in the second step of the method either a proportionally greater decrease in rate or a larger H_L is necessary to restore the lost moment. Further heating again requires either a further increase in τ_L or an increase in H_L .

It is possible for alteration to result in an increase in moment; in that event H_L or τ_L must be continually decreased. If when alteration is in progress the moment should decrease with time it can sometimes be identified as follows: if H_L is chosen so that the moment shows the characteristic decrease followed by an increase when alteration is absent at lower temperatures, the moment will decrease when alteration begins. Then it is possible for the moment to decrease then increase and then decrease again, due to alteration. An example of this behaviour is shown in Fig. 3.

Probably the most surprising aspect of the alteration is that it has manifested itself at temperatures as low as 210 °C. Also, of 18 samples of Attic pottery 15 showed evidence of alteration at temperatures below 250 °C.

Most investigators obtain the ratio of the ancient to laboratory fields from the slope of a 'best' straight line drawn through the points in a plot of the natural remanent moment against the moment introduced by a laboratory field at successively higher temperatures. Unfortunately, in most cases it is difficult to demagnetize the sample sufficiently to draw a meaningful line unless temperatures above 300 °C are used, where the present results indicate that alteration may be present. In the event that

those points are displaced from their true positions by increasing amounts the slope will be wrong.

The effect of cooling rate has been thought to introduce a correction which would be a constant fraction of the ancient field, assuming that the ancient cooling rate was within a factor of 2 or 3 for all pottery (at least those fired in the same area). Unfortunately the preliminary results obtained with Attic pottery indicate rather more variability. One advantage of the present technique is that measurements at low temperatures yield the ancient field. Thus, provided alteration does not begin before all viscous moments are removed, a value for H_A can always be obtained. If an accurate value of H_A is not required it is possible to assume a value for τ_A , in which case one, or at most two, temperatures are all that is necessary.

Finally, the previous measurements⁴ on Attic pottery should be repeated, as they use values obtained above 300 °C. These measurements are in progress, and the results will be published in due course. Preliminary results indicate that the shape of the curve of intensity plotted against time has not changed radically, but that the values of intensity have decreased by ~15–25%.

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Sm–Nd dating and cooling history of Scourian granulites, Sutherland

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The Scourian gneisses of the Lewisian complex of north-west Scotland have been the subject of many geochronological investigations. Sm–Nd whole-rock measurements suggest that the protoliths of the Lewisian complex differentiated 2.92 ± 0.05 Gyr ago from an approximately chondritic mantle¹. Rb–Sr and Pb–Pb whole-rock ages between ~2.8 and 2.6 Gyr reflect subsequent depletion in Rb and U (refs 2, 3) which has generally been associated with granulite facies metamorphism. Zircons which are thought to have crystallized during this metamorphism have also given U–Pb ages of 2.66 Gyr (ref. 4). This granulite facies metamorphism, involving pressures in excess of 10 kbar and peak temperatures estimated between 820 ± 50 °C (ref. 5) and $1,250$ °C (refs 6, 7), affects a terrain including supracrustal rocks⁷ and thus implies a major tectonic event. We report here the first geochronological data measured on mineral assemblages that have also provided estimates of the conditions of metamorphism. Such measurements are possible because the major minerals of granulite facies assemblages show an unusually favourable spread in Sm/Nd ratios; garnet, especially, is strongly enriched in Sm (ref. 8). Data for three garnetiferous basic gneiss assemblages suggest that closure of the Sm–Nd system occurred significantly later than the peak of the metamorphism.

The textures of Scourian basic gneisses are complex and it has been suggested that three stages of granulite facies mineral growth can be recognized^{6,9}. The earliest assemblage consists of garnet and clinopyroxene with minor plagioclase and hornblende. During the second stage garnet broke down to form coronas of plagioclase, orthopyroxene and magnetite. Subsequently regrowth of garnet, especially rimming grains of orthopyroxene and magnetite, occurred. Locally, much coarser (up to 150 mm) aggregates of garnet and clinopyroxene occur within the garnetiferous basic gneisses; it has been suggested that these are the result of exsolution from an Fe–Al rich

amphibole¹⁰. Sample 3966 'coarse' is an example of these aggregates, occurring in a typical basic gneiss represented by 3966 'fine'. There is a sharp contact between the two assemblages. The third sample, 40117, is also a typical garnetiferous basic gneiss. All three assemblages show, to varying degrees, the three stages of mineral growth noted above.

The results of the isotopic analyses are given in Table 1 and are plotted on Sm–Nd isochron diagrams in Fig. 1. In each case the data show some scatter in excess of analytical error but the three calculated ages are similar. 40117 gives an age of 2.49 ± 0.12 Gyr with an initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.50944 ± 0.00016 , 3966 'fine' gives 2.49 ± 0.03 Gyr with an initial ratio of 0.50949 ± 0.00009 and 3966 'coarse' gives 2.49 ± 0.03 Gyr with an initial ratio of 0.50973 ± 0.00008 . The garnet and clinopyroxene in the analysed mineral separates are from the earliest stage of mineral growth whereas the other analysed mineral separates also include grains from the later stages. The isotopic relationships between the minerals show that the different stages of mineral growth cannot be resolved with the present data. Moreover, the earliest formed minerals, garnet and clinopyroxene, systematically give ages that are up to 0.05 Gyr younger, rather than older, than those calculated for the complete mineral assemblage; thus the excess scatter in the calculated errorchrons does not simply reflect differences in the age of formation of the various minerals.

The consistency of the ages from all three samples may reflect a relatively short overall duration for the granulite facies metamorphism, or they may date closure of the Sm–Nd system at some stage in the post-metamorphic cooling. The latter is strongly supported by the interpretation of the 2.66-Gyr zircon age as the result of zircon growth during granulite facies metamorphism⁴, and by 2.56 Gyr Rb–Sr ages on pegmatites¹¹ which intruded the basic gneisses after the granulite facies metamorphism¹². Theoretical closure temperatures can be calculated from experimentally determined diffusion coefficients¹³, but such data are only available for Sm in grossular and pyrope at temperatures between 1,100 and 1,500 °C (see ref. 14 and

Table 1 Sm–Nd isotopic analyses of minerals from three samples of garnetiferous basic gneiss

Mineral	Sm (p.p.m.)	Nd (p.p.m.)	$^{147}\text{Sm}/^{144}\text{Nd}$ $\pm 2\sigma$	$^{143}\text{Nd}/^{144}\text{Nd}$ $\pm 2\sigma$
40117, Scourie Mhor (GR 142442)				
GT	1.64	0.94	1.0696 ± 20	0.52690 ± 22
	1.68	0.98	1.0529 ± 134	0.52694 ± 3
CPX	3.47	11.65	0.1833 ± 3	0.51279 ± 15
OPX	0.15	0.61	0.1516 ± 40	0.51210 ± 8
PLAG	0.21	1.44	0.0889 ± 1	0.51096 ± 5
MT	0.44	1.50	0.1824 ± 1	0.51239 ± 3
	0.47	1.64	0.1748 ± 1	0.51262 ± 6
HB	6.91	23.98	0.1772 ± 1	0.51233 ± 3
3966 'coarse', north Scourie Bay (GR 152452)				
GT	1.70	1.31	0.7960 ± 19	0.52277 ± 7
	1.75	1.36	0.7932 ± 4	0.52273 ± 5
CPX	0.63	2.36	0.1647 ± 7	0.51247 ± 3
PLAG	3.11	19.11	0.1000 ± 3	0.51131 ± 4
MT	0.25	1.90	0.0826 ± 4	0.51157 ± 34
3966 'fine', north Scourie Bay (GR 152452)				
GT	1.91	1.52	0.7696 ± 5	0.52215 ± 3
	1.97	1.59	0.7614 ± 4	0.52202 ± 5
CPX	4.11	15.33	0.1650 ± 13	0.51224 ± 3
	4.02	14.89	0.1661 ± 2	0.51231 ± 5
PLAG	0.39	2.96	0.0813 ± 5	0.51092 ± 3
HB	7.40	29.87	0.1523 ± 6	0.51193 ± 2

GT, garnet; CPX, clinopyroxene; OPX, orthopyroxene; PLAG, plagioclase; MT, magnetite; HB, hornblende. Most of the analytical uncertainty on the $^{147}\text{Sm}/^{144}\text{Nd}$ ratio is from isotope dilution analysis of the Sm. For many of these analyses, Sm was run on a single Ta filament. Greater precision ($\pm 0.05\%$) has since been obtained by running Sm on a triple filament assembly with a central Re and side Ta filament. The high concentrations in plagioclase from sample 3966 'coarse' reflect the presence of small discrete grains of apatite within the sample.

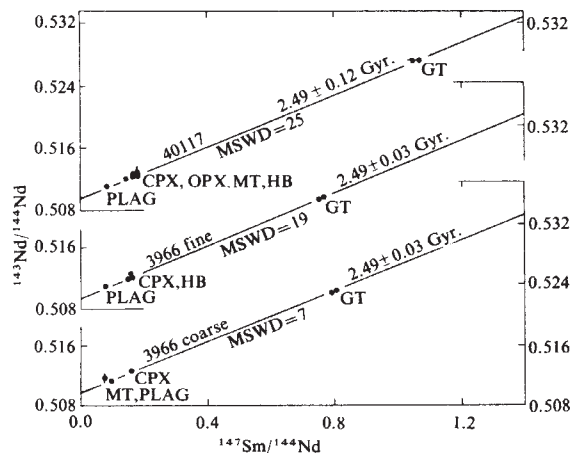


Fig. 1 Sm-Nd diagrams. $\lambda^{147}\text{Sm} = 6.54 \times 10^{-12} \text{ yr}^{-1}$. The regression errors have been multiplied by $\sqrt{\text{MSWD}}$ to make allowance for the excess scatter. The magnetite (MT) data were omitted from the regression analysis of sample 3966 'coarse'.

Table 2). To apply these data to Nd isotopes it must be assumed that (1) the diffusion behaviour of Nd is similar to that of Sm, (2) extrapolation from the experimental to geological temperature ranges using the Arrhenius equation is valid, and (3) the difference in diffusion coefficients between pure grossular and pure pyrope encompasses the value appropriate to the dated garnets ($\text{Alm}_{1.6}\text{Py}_{0.9}\text{Gross}_{0.5}$). Consideration of anion porosity¹⁵ suggests that Sm diffusion coefficients should increase systematically across the solid solution series from pyrope to grossular and for the end members this is confirmed by the experimental data (Table 2). Because of the variation in activation energy with composition, at the temperatures of interest it is expected that D_{pyrope} will be greater than $D_{\text{grossular}}$; closure temperatures based on these values should bracket the closure temperature for the analysed garnets.

Calculated closure temperatures are given in Table 2. The result for pyrope does not exceed 600 °C even in conditions of very rapid cooling (100 °C Myr^{-1}); for grossular the corresponding value is 775 °C. In each case a slower cooling rate reduces the calculated closure temperatures. These temperatures are below even the lowest estimated conditions at the peak of the granulite facies metamorphism (820 °C)⁵ so that the available diffusion data also suggest that the measured ages should be interpreted as cooling ages.

While the concordant Sm-Nd ages indicate a close approach to isotopic equilibrium within the individual assemblages, that is between grains of 1–5 mm diameter, the initial ratios for the two assemblages from 3966 are significantly different, thus showing that the scale of diffusion 2.49 Gyr ago was $\ll 100$ mm. The higher initial ratio of 3966 'coarse' also shows that the higher

bulk Sm/Nd ratio of this assemblage was established appreciably earlier than 2.49 Gyr. Figure 2 shows the evolution of $^{143}\text{Nd}/^{144}\text{Nd}$ with time for 3966. Because 3966 'coarse' contributes insignificantly to the Sm-Nd mass balance for the whole sample, the pre-metamorphic evolution of this sample is approximately represented by the growth line for 3966 'fine'. The fact that this line intersects the error ellipse for the Lewisian whole-rock suite¹ supports this interpretation. During metamorphism at $\sim 2.62 \pm 0.12$ Gyr, 3966 'coarse' was formed as an isolated sub-system with higher Sm/Nd and its $^{143}\text{Nd}/^{144}\text{Nd}$ began to grow at a correspondingly faster rate. In view of the difficulties in estimating modes in coarse-grained rocks, this estimate of the time of segregation is very tentative, although the intersection age agrees well with the 2.66 Gyr age of metamorphic zircons⁴.

The relation between the isotopic ages and the temperature history based on conventional geothermometry is not as clear as the above discussion would suggest. Temperature estimates for the metamorphic peak have been based on feldspar thermometry⁶, pyroxene exsolution^{6,7} and Fe-Mg binary exchange equilibrium between garnet and clinopyroxene⁵. In conditions of slow cooling, these reactions are also likely to continue after the metamorphic peak, like diffusion of Nd isotopes, until the relevant closure temperature is reached and the compositions of the minerals are frozen in. Realistic closure temperatures for the metamorphic reactions are difficult to estimate as the commonly used reactions involve major components whose diffusion rates may be strongly coupled with diffusion of other major components which do not directly participate in the reaction¹⁶.

For binary exchange reactions there may be further problems where the two minerals involved have different closure temperatures. Consider the Fe-Mg exchange between garnet and clinopyroxene. Apparent equilibration temperatures for this reaction in rocks from Scourie are typically between 750 and 850 °C (refs 5, 10, and F.J.H. unpublished data on the dated samples). Experimental data on the interdiffusion of Fe and Mg in garnet¹⁷ (Table 2) indicate a closure temperature of 725 ± 30 °C for 5 °C Myr^{-1} cooling rate. Diffusion is likely to be slower in clinopyroxene which would therefore close at a higher temperature than garnet. Below the closure temperature of clinopyroxene, garnet may potentially exchange with other Fe-Mg minerals, for example orthopyroxene in the coronas around garnets. This would result in a more Fe-rich garnet and lead to erroneously low temperature estimates. The consistency of temperatures calculated from garnet-clinopyroxene pairs and

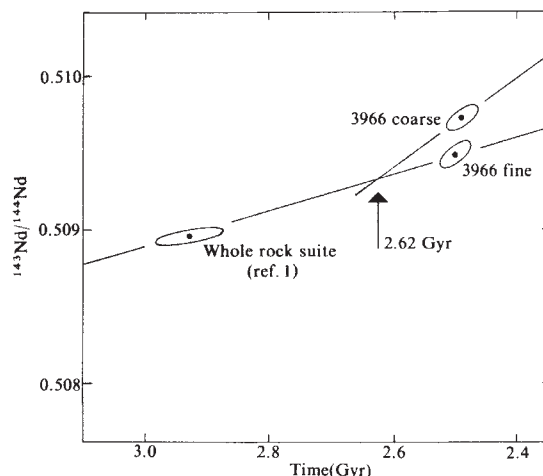


Fig. 2 Nd isotopic evolution in sample 3966. The data points for 3966 'coarse' and 3966 'fine' are the ages and initial ratios of the respective mineral isochrons. The data point for Lewisian whole rocks is the age and initial ratio of the whole-rock isochron from ref. 1. The 95% confidence ellipses were calculated following ref. 18. The lines representing the growth of $^{143}\text{Nd}/^{144}\text{Nd}$ with time were calculated from estimated 'whole-rock' Sm/Nd ratios for 3966 'coarse' and 3966 'fine', based on modal analyses and the Sm and Nd contents of the analysed minerals.

Table 2 Diffusion in garnet

Mineral	Species	D_0 ($\text{m}^2 \text{ s}^{-1}$)	E (kJ mol^{-1})	Ref.	T_c^*
Pyrope	Sm	2.6×10^{-12}	140	14	480
Grossular	Sm	4.3×10^{-4}	331	14	700
Almandine-pyrope	Fe-Mg inter-diffusion	6.11×10^{-4}	344	17	725

Diffusion rate, D , at temperature T is given by the Arrhenius relationship:

$$D = D_0 \exp(-E/RT)$$

where R is the gas constant. Theoretical closure temperature, T_c , is related to the cooling rate $\dot{T}$ by:

$$E/RT_c = \ln(-A D_0 R T_c^2 / E \dot{T} a^2) \quad (\text{ref. 13 equation (11)})$$

where a is the radius of the grain; A is a geometric factor, equal to 55 for radial diffusion in a sphere.

* Closure temperature calculated for a cooling rate of 5 °C Myr^{-1} .

likely closure temperatures perhaps indicates that this is not a major problem in the rocks considered here, although it may explain some of the lower temperatures that have been reported⁵. From the above discussion we know that binary exchange thermometers will not record the peak metamorphic temperatures if the relevant closure temperatures are exceeded. Thus neither the estimate of 820 °C based on such reactions⁵ nor the higher estimate of 1,250 °C (refs 6, 7), which was criticized on other grounds by Rollinson⁵, are well founded and the peak conditions of Scourian metamorphism remain uncertain.

From the estimated closure temperatures for Nd and Fe–Mg in garnet it is suggested that the temperatures recorded by the garnet–clinopyroxene geothermometer date from a time before 2.49 Gyr. After the metamorphic peak close to 2.66 Gyr, the Scourian complex cooled at such a rate that temperatures

did not drop below ~600 °C until after 2.49 Gyr.

Our data confirm the potential of Sm–Nd dating in basic granulites and the ability to determine metamorphic conditions and isotopic ages on the same samples. However, diffusion of rare earth elements between mineral grains is sufficiently rapid in granulite facies conditions to prevent the preservation of Sm–Nd crystallization ages. Also the uncertain relationships between closure of different geochemical systems is a fundamental limitation on our ability to determine metamorphic conditions.

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Asphalt in carbon-14-dated archaeological samples from Terqa, Syria

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The presence of fossil fuel compounds in archaeological samples giving ages much higher than expected has long been suspected but never proved by appropriate chemical analyses. An excessively high conventional ¹⁴C age was found in an archaeological charcoal sample from Terqa, Syria¹ (S313, Table 1). Its ¹⁴C age of 28,700 yr BP was at variance with the archaeological context, and with the ¹⁴C age of several other samples¹ (such as S283, Table 1) obtained from the same area and expected to date from ~3000 BC or younger. The inconsistency can be explained by assuming contamination with geologically old material—either industrial petroleum products or ancient asphalt. We report here organic geochemical investigations of the sample S313 and of sample S267, which gave a ¹⁴C age ~2,000 yr too old. A third charcoal sample (S283) from the same area, whose ¹⁴C age was consistent with its known historical age, and an asphalt sample found stuck to the bottom of a goblet from another site in the area dated at 1700 BC on typological grounds, were also analysed. Similar studies of used crankcase oils from motor vehicles, weathered 0–2 yr, and of hydraulic transmission fluid show that modern motor oil cannot be the contaminant at this excavation site. Our data clearly indicate that ancient asphalt must be the source of contamination. Caution should be exercised, therefore, in interpreting ¹⁴C dates of archaeological samples from areas containing asphalt or other fossil fuel deposits.

Fine charcoal samples were collected by D. Berry from and around an ancient dumpsite outside the ancient city wall of Terqa, Syria and stored in glass jars (see ref. 1 for details). Obvious extraneous matter, such as rootlets, was removed; the samples were converted to acetylene via lithium carbide² and counted in stainless-steel gas proportional β-detectors for ¹⁴C (Table 1).

The samples were Soxhlet-extracted successively with methanol and toluene/methanol (3:7 v/v) for 100 h. The extract was separated into hexane-insoluble (asphaltenes) and

hexane-soluble (lipid or bitumen) fractions. The latter was fractionated into aliphatic and aromatic hydrocarbons and resins by silica gel column chromatography⁴. Samples S313 and S267 had asphaltenes and S267 also had traces of elemental sulphur (Table 1). Sample S283 had no asphaltenes, but a considerable amount of elemental sulphur and resinous material characteristic of wood. The fractions were analysed by gas chromatography (GC) and GC–mass spectrometry. Sample S313 was ¹⁴C tested again after exhaustive extraction with toluene/methanol (3:7 v/v) and methylene chloride. After rinsing with 1 l of acetone to remove other organic solvents, the material was analysed for ¹⁴C measurements². The ¹⁴C age determined was not significantly different from that found previously for the unextracted sample (Table 1).

The gravimetric (Table 1) and GC analyses revealed that the greater the discrepancy in age, the larger is the content of asphaltenes and hydrocarbons. The alkane profile of the control sample (S283) with correct ¹⁴C age was very different from those of the other two samples (Fig. 1, S267 not shown).

The normal alkanes in sample S283 ranged from C₁₅ to C₃₁ overlying a broad unresolved complex mixture (Fig. 1). Pristane and phytane are almost equally abundant. The carbon preference index (CPI) is ~2.0, indicative of immature hydrocarbons derived from higher plant waxes⁵. Triterpanes are present in minor amounts and are mainly 17α(H), 21β(H)-hopanes. The lipids in this charcoal sample appear to be of mixed secondary origin from biogenic sources and traces of petroleum-like products seem to have been leached into the area by water over thousands of years. The presence of a few pyrolytic arenes⁶ is consistent with such an input. The alkanes in samples S313 and S267 are predominantly cyclic triterpanes with minor amounts of steranes. Normal alkanes are found only in trace amounts (much less than in crude oils). The triterpanes consist mainly of a homologous series of 17α(H), 21β(H)-hopanes and the extended hopanes (≥C₃₁) occur as their two diastereomers (R and S at position 22) in approximately equal abundance⁷. In fact, the ratio of the first eluting epimer (22S) to the second eluting epimer (22R) in the C₃₁–C₃₅ hopane series is ~1.4 (measured with baseline GC resolution), consistent with mature ancient bitumen⁸. The aromatic fraction exhibits an unresolved hump over the entire boiling range, indicating heavy weathering of the lipid fraction. The low n-alkane content, such as that found in sample S283, could be masked in the other two samples which contain relatively higher concentrations of triterpanes from the presumed (fossil fuel) contamination.

Possible sources of oil contamination in the area are crankcase oil from motor vehicles, although the samples were retrieved from a depth of 2 m below the surface of an area with little or no

Table 1 Organic geochemical results for archaeological samples from Terqa, Syria

Sample	Conventional ^{14}C age (BP)	Elemental sulphur* ($\mu\text{g per g}$)	Asphaltenes* ($\mu\text{g per g}$)	Hexane-soluble lipids* ($\mu\text{g per g}$)	Aliphatic fraction* ($\mu\text{g per g}$)	Aromatic fraction* ($\mu\text{g per g}$)	Asphaltenes			
							H/C	$\delta^{13}\text{C}$ (‰ PDB)	$\delta^{18}\text{O}$ PDB	δD (‰ SMOW)
Charcoal S283	4,110 $\pm$ 70	~15	—	144	41	53	—	—	—	—
Charcoal S267	5,700 $\pm$ 100	Trace	900	1,440	260	86	1.27	-27.61	-15.58	-60.9
Charcoal S313	28,700 $\pm$ 1,100 28,200 $\pm$ 1,300†	—	5,300	14,560	2,790	1,820	1.03	-27.61	-15.88	Lost
Asphalt from goblet	—	—	104,000	43,000	4,860	2,410	1.23	-27.95	-15.58	-60.0

1 σ statistical counting errors are given for the conventional BP dates. The calibrated dates in centuries can be obtained using the table in ref 3. The age of the charcoal samples expected from stratigraphy is 3000 BC. PDB, Pee Dee belemnite. SMOW, standard mean ocean water. La Jolla Laboratory numbers in sequence of increasing ^{14}C are: LJ 5052, LJ 4823 and LJ 5031.

* Gravimetric determination. † After exhaustive extraction with organic solvents.

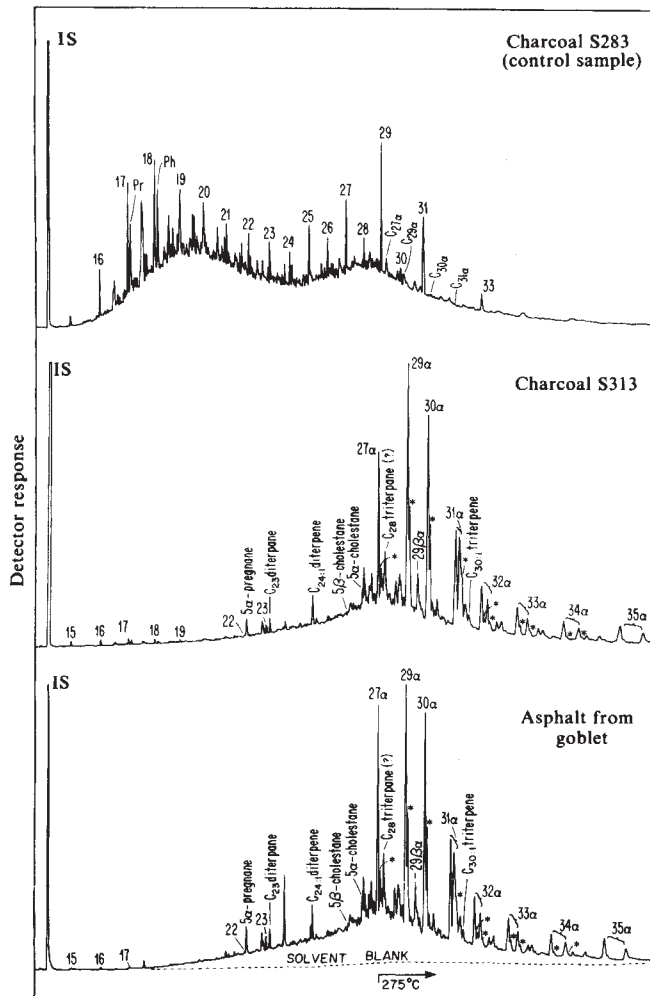


Fig. 1 Gas chromatograms of aliphatic fraction from charcoal and asphalt samples from Terqa, Syria. A Hewlett-Packard 5840A gas chromatograph equipped with a fused silica capillary column (SP-2100, 25 m, 0.2 mm i.d.) was used. The column was temperature-programmed for 35–275 °C_{iso} at 4 °C per min. A 30 m, SE-54 (J&W) column of the same specifications was used for GC-MS analysis on a Finnigan model 4000 quadrupole mass spectrometer interfaced with a Finnigan Model 9610 gas chromatograph. The mass spectra data were processed with a Finnigan Inco Model 2300 data system. *n*-Alkanes were identified by their retention times. Di- and triterpenoids were identified from *m/z* 191 mass chromatograms. Triterpanes $\geq \text{C}_{31}$ occur as *R* and *S* diastereomers. IS, internal standard (hexamethylbenzene); numbers (except when followed by α or β) refer to *n*-alkanes; Pr, pristane; 27 α , 29 α , and so on refer to 17 α (H), 21 β (H)-hopanes with the designated carbon numbers; 29 $\beta\alpha$ is a C₂₉ moretane (17 β (H), 21 α (H)). * Refers to the homologous series of methylated hopanes. Charcoal sample S267 had the same GC profile as S313 and therefore is not shown here.

traffic until a few years ago, and ancient asphalt. The area on the Euphrates in Syria contains asphalt⁹.

We analysed the hydrocarbon fractions of fresh hydraulic fluid and crankcase oil, fresh and weathered for ~1 month to 2 yr. The crankcase oil was collected from underneath impounded cars and the period of weathering of the spilled oil from the cars is documented. The GC profiles of the hydrocarbons in the oils (Fig. 2) have a major unresolved hump at C₂₀–C₃₀ with a homologous series of *n*-alkanes and branched and cyclic hydrocarbons, in contrast to the contaminated charcoal samples which are depleted in *n*-alkanes. If the contaminant were crankcase oil weathered only since cars began operating in this region (20–40 yr), then almost total degradation of *n*-alkanes would be impossible. This can be surmised if we compare the fresh oil to the one weathered over two years (also heated to a certain extent in the car). The maximum temperature to which crankcase oil is normally heated is ~205 °C (250 °C in diesel engines, D. Godfrey, personal communication), which may not be high enough to pyrolyse all the *n*-alkanes^{10,11} in the time during which the oil is heated in the car. Also, the stratigraphy at the sample locations showed no streaks of spilled oil from the surface to the 2 m depth of the samples. These observations preclude automobile oil from being the contaminant.

The only other possible source seems to be ancient, indigenous asphalt. Modern asphalt seems an unlikely candidate as there were no paved roads near the excavation site. An asphalt sample from a goblet in the area had the same *n*-alkane (trace amounts) and triterpenoidal distributions as did the two contaminated samples (Fig. 1). The H/C ratio and $\delta^{13}\text{C}$ data of the asphaltenes from the samples (Table 1) are typical of data reported for asphalts^{12–14}. The close agreement in the stable isotopic data (of carbon, oxygen and hydrogen obtained according to ref. 15) of the asphaltenes also suggests a common source of asphalt in the contaminated samples and the asphalt from the goblet, that is, indigenous asphalt.

The nonextractable residue, after exhaustive solvent extraction, gave almost the same ^{14}C age as the original sample (Table 1), suggesting that the unusually old age is largely due to the insoluble pyrobitumen¹⁶ that could have originated from the asphalt. (Modern motor oil would probably be completely extracted by organic solvents used on sample S313, in which case the residue should have given a comparable age to that of other syngenetic samples in the area.)

In the third millennium in Syria, the asphalt used for sealing cracks in ceramic vessels¹⁷ and fastening tool heads onto wooden handles⁹ was probably melted by heating^{18,19}. Frequent reheating, coupled with weathering, might have altered most of the asphalt into pyrobitumen. The residual asphalt which did not undergo complete thermal alteration could contain a triterpenoidal profile similar to the one reported here. At elevated temperatures (~375 °C) straight-chain alkanes decompose¹⁰, whereas tricyclic diterpanes and pentacyclic triterpanes survive²⁰. Indeed, the triterpenoidal distribution in the contaminated samples and asphalt are very similar to those of

pyrolysates from kerogen matrices²⁰. These pyrolysates have lesser amounts of steranes and moretanes compared with triterpanes and the $C_{29}\alpha$ hopane is more abundant than the $C_{30}\alpha$ homologue, as is observed here (Fig. 1). A minor series with m/z 205, analogous to the major hopane series, is also present, indicating an extra methyl group in the A or B ring. 3-Methylhopanes have been detected in bacteria²¹ and the presence of the methylated hopanes implies microbial residues probably involved in the synthesis of petroleum hopanes²². Water washing and biodegradation of crude oil in reservoirs also could remove n -alkanes and isoprenoids and lead to a similar distribution of triterpenoids in the resultant asphalt²³ as these compounds do not seem to be significantly affected by bacterial degradation^{23,24}. It is not surprising, therefore, to see the biomarker steranes and di- and triterpanes (derived from the thermally altered and probably biodegraded indigenous asphalt) in the charcoal samples.

A broken storage pot of asphalt dumped in refuse could easily have contaminated some of the charcoal samples excavated from the ancient dumpsite. It is also feasible, that some of the native asphalt might have undergone thermal alteration by its proximity to the fireplace from which the charcoal samples could have originated. It is not known whether the asphalt was used as a fuel.

According to the ^{14}C data, pyrobitumen from ancient asphalt has contributed as much as 95.5% and 18% to the total carbon in samples S313 and S267 respectively. This large amount explains why, after removing the solvent solubles, a younger ^{14}C age was not observed for S313. The extractable material was much lower in concentration relative to the non-extractables.

This is the first organic geochemical study to verify contamination in ^{14}C dated archaeological samples, which could account for much older apparent ages than expected. Chronologies based on ^{14}C dates of archaeological materials containing fossil organic carbon in the absence of confirmatory molecular analysis should be accepted with caution. Similar organic geochemical analyses of a few samples (^{14}C dated) from Jericho and Jarmo (northern Iraq) where traces of asphalt have been found on ancient stone tools¹⁸ are in progress.

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O-sulphated Leu-enkephalin in brain

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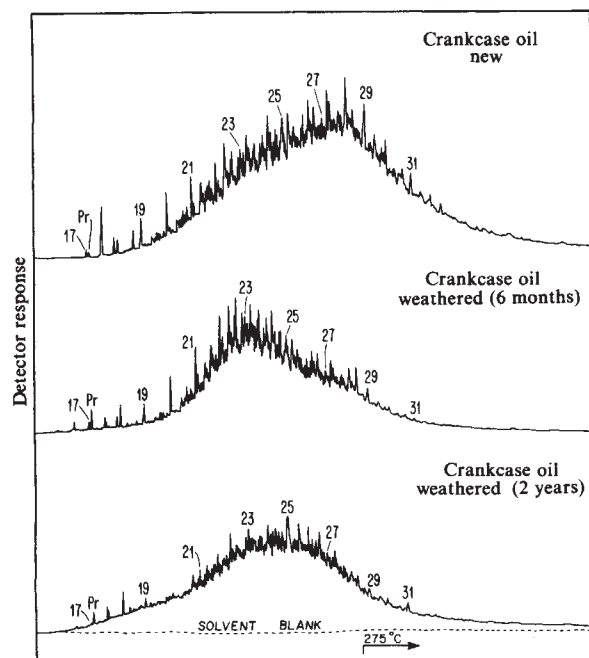


Fig. 2 Gas chromatograms of aliphatic fraction from crankcase oils. Experimental details as for Fig. 1. Numbers refer to n -alkanes; Pr, pristane.

The enkephalins appear to share a common precursor which is distinct from pro-opiocortin^{1,2}. The adrenal proenkephalins have molecular weights (M_r s) in the range 5,000-30,000 with the largest species containing six copies of Met-enkephalin to one of Leu-enkephalin³. The brain proenkephalins appear to differ in that the molecular weights are in the range 5,000-90,000 and digestion of the largest protein with trypsin and carboxypeptidase B generates approximately equal proportions of Met- and Leu-enkephalin⁴. During our studies of brain proenkephalin we noted the generation of enkephalin-immunoreactive species that were chromatographically distinct from Met- and Leu-enkephalin. Here we identify one of these species as O-sulphated Leu-enkephalin and suggest possible roles for sulphation in the metabolism of opioid peptides.

Digestion of the striatal proenkephalin fraction ($M_r > 5,000$) with trypsin yielded a mixture of enkephalin-like immunoreactive and bioactive opioid species; these were separated by

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high-voltage paper electrophoresis (HVPE) at pH 6.5 into basic, neutral and acidic species. The neutral peptides were analysed by reverse-phase HPLC using antisera directed against Met- and Leu-enkephalin, which showed that the two peptides were present in a ratio of 2:1. The precursor pool therefore contains extreme C-terminal enkephalin sequences with the Leu-enkephalin form predominating. Treatment of the basic peptide fraction with carboxypeptidase B yielded a further mixture of Met- and Leu-enkephalin with the former predominating although we were unable to determine an absolute ratio because of the presence of other unidentified immunoreactive species. Treatment of the acidic fraction with carboxypeptidase B resulted in an increase in Leu-enkephalin immunoreactivity but this activity still moved towards the anode at pH 6.5 and was found to possess a negative charge even at pH 1.9. Isoelectrophoretic analysis confirmed the presence of immunoreactive and bioactive opioid peptides with *pI* values of 3.5 to <2.5, that is, beyond the range of our ampholyte system. The peptides derived from trypsin and carboxypeptidase B digestion were subjected to HVPE both before and after treatment with neuraminidase, phosphatase and arylsulphatase. Treatment with the first two enzymes had no discernible effect on the mobility or immunoreactivity of the acidic fraction; however, arylsulphatase treatment caused a marked increase in Leu-enkephalin immunoreactivity which now co-migrated with marker enkephalin. When the acidic peptide fraction derived from trypsin digestion alone was reacted with arylsulphatase, the products were found to carry a positive charge at pH 6.5.

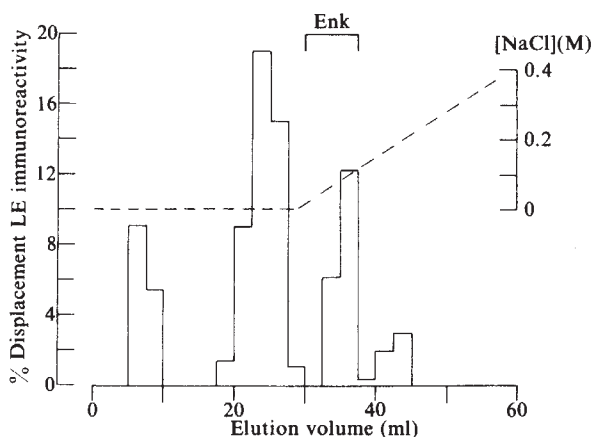


Fig. 1 Separation of tryptic opioid peptides by DEAE-ion-exchange chromatography. Mouse striatum was extracted with 10 ml g^{-1} of 1M acetic acid adjusted to pH 1.9 with HCl. The soluble proteins were passed through a Sephadex-G25 column and the excluded volume was collected and lyophilized. This protein fraction was dissolved in 50 mM Tris-HCl pH 8, incubated with trypsin (1 mg per 100 mg protein) for 4 h, then passed through a 400 mg Poropak-Q column and after a water wash the absorbed peptides were eluted with ethanol. The dried ethanol eluate was dissolved in 50 mM Tris-HCl, pH 9.25 and applied to a $20 \times 0.7 \text{ cm}$ DEAE-Sephadex (A-25) column. The column was first developed with 28 ml of 50 mM Tris-HCl pH 7.3, at which point a linear salt gradient was started. Aliquots of each 2.5-ml fraction, corresponding to 3 mg of starting tissue, were immunoassayed with both anti-Leu-enkephalin-antisera (LE1, 5% cross-reactivity with Met-enkephalin) and anti-Met-enkephalin-antisera (ME-T1, <0.1% cross-reactivity with Leu-enkephalin). The first two peaks, 0.6 and 1.2 pmol Met-enkephalin-equivalents, represent basic peptide fractions and only reacted with ME-T1. The third peak (0.33 pmol) corresponds to the elution position of authentic enkephalin and contained Leu- and Met-enkephalin in a 2:1 ratio. The fourth peak (0.11 pmol Leu-enkephalin (LE) equivalents) only reacted with LE1 and carried a negative charge at pH 6.5. Treatment of this peak with carboxypeptidase B gave a slight increase in immunoreactive material carrying a negative charge at pH 1.9. (Note that cross-reactivity of LE1 with Arg or Lys C-terminal-extended Leu-enkephalin is only slightly less than that of *O*-sulphated-Leu-enkephalin.)

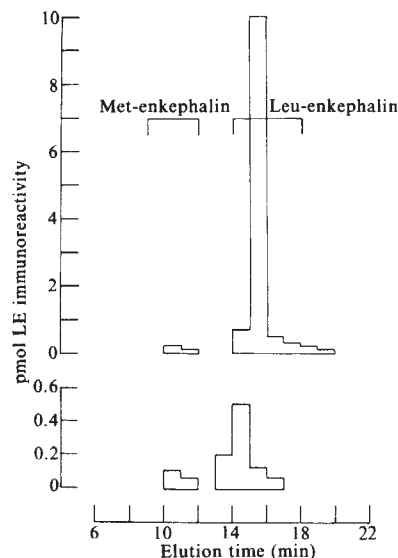


Fig. 2 Reverse-phase HPLC analysis of acidic Leu-enkephalin (LE)-immunoreactive peptide. Sheep striatum (70 mg protein) was extracted and treated with trypsin as in Fig. 1 and the acidic Leu-enkephalin-immunoreactive peak isolated by DEAE chromatography. This material was incubated at 37 °C for 2 h with 10 μg of carboxypeptidase B in 50 mM Tris-HCl, pH 8. One half of the sample was then incubated with arylsulphatase (0.5 unit) from *Patella vulgata* (200 mM sodium acetate, pH 5 for 2 h). The products of these digestions were absorbed on Poropak-Q and then chromatographed on a $250 \times 4 \text{ mm}$ Partisil-10-ODS-2 column in isocratic conditions (100 ethanol/250 water/3.5 acetic acid by vol) at 0.8 ml min^{-1} . The lower panel shows the elution profile of LE1 immunoreactivity after trypsin and carboxypeptidase treatment, the upper panel after the additional arylsulphatase treatment.

Gradient elution of the tryptic digest on Sephadex-DEAE revealed the presence of four peaks of immunoreactivity (Fig. 1). Peaks 1 and 2 reacted strongly with anti-Met-enkephalin antibodies and were strongly basic. Peak 3 corresponded to the elution position of authentic Met- and Leu-enkephalin, the greater affinity for anti-Leu-enkephalin antisera reflecting the preponderance of Leu-enkephalin generation after trypsin digestion. Peak 4 corresponds to the acidic fraction detected by HVPE and showed a much greater affinity for anti-Leu- rather than for anti-Met-enkephalin antisera. Peak 4 was divided and one fraction treated with carboxypeptidase B, the other with carboxypeptidase B and arylsulphatase. These fractions were chromatographed on a Partisil-10-ODS (octadecyl silane) HPLC column (Fig. 2). Immunoassay of the effluent revealed the presence of a small Leu-enkephalin-immunoreactive peak in the carboxypeptidase B-treated fraction, this peak being close to, but not coincident with, an authentic Leu-enkephalin sample. The arylsulphatase-treated fraction yielded a much larger Leu-enkephalin-immunoreactive peak coincident with authentic Leu-enkephalin. Identical results were obtained with protein extracts of both mouse and sheep striatum.

These results indicated the presence in our protein fraction of a sulphated Leu-enkephalin sequence flanked by basic amino acids. Trypsin digestion alone should liberate a sulphated Leu-enkephalin sequence containing C-terminal Arg or Lys residues and carrying an overall negative charge due to the presence of *O*-sulphate. Treatment with arylsulphatase should change the overall charge to +1 due to the loss of sulphate while treatment with carboxypeptidase B and arylsulphatase yields a Leu-enkephalin-like peptide having no overall charge at pH 6.5. We cannot exclude the possibility that some sulphated Leu-enkephalin sequences might exist at the extreme C-terminal of proenkephalin but the above evidence supports an internal location for most of the material.

In view of the small amounts of natural material available, the *O*-sulphate ester of Leu-enkephalin (LES) was prepared and its

properties compared with those of the material derived from enzymatic digestion of striatal proenkephalin. LES was prepared by coupling *N*-*t*-butoxycarbonyl-1-tyrosine-*O*-sulphate barium salt with glycylglycyl-1-phenylalanyl-1-leucine *t*-butyl ester. The final purified product was homogeneous in five TLC and two HPLC systems and gave amino acid ratios, after aminopeptidase M digestion, of Tyr(SO₃H) (1): Gly (1.99): Phe (0.97): Leu (1): Tyr (0), (J.S.M. and C. F. Hayward in preparation). LES caused a dose-dependent, naloxone-reversible inhibition of both the mouse vas deferens and guinea pig ileum but had only 0.0001 times the activity of Leu-enkephalin in these assays. Its cross-reactivity with our anti-Met- and anti-Leu-enkephalin antisera was <0.1% and 5% respectively. LES has no intrinsic opiate receptor activity as a small proportion can undergo desulphation during bioassay. Incubation of LES with arylsulphatase for 2–4 h caused a maximal conversion of 20–30% of the material to Leu-enkephalin within 2 h. An alternative method of desulphation was developed based on the observation that exposure of LES to anhydrous trifluoroacetic acid (TFA) during its synthesis causes a rapid loss of the sulphate moiety. Incubation of dried samples of LES with anhydrous TFA (50 µg LES in 500 µl TFA) at 37 °C resulted in complete conversion to Leu-enkephalin within 80 min as judged by HPLC. No other product was detected by HPLC nor was there any alteration of Leu-enkephalin activity.

Synthetic LES and our acidic Leu-enkephalin-immunoreactive fraction had identical mobilities in several chromatographic systems including silica-gel TLC, Partisil-10-anion-exchange HPLC and reverse-phase HPLC. Synthetic LES eluted between Met- and Leu-enkephalin on the Partisil-10-ODS system with overlap between Leu-enkephalin and LES. This was consistent with the result obtained before the synthetic material was available (Fig. 2). Complete separation of LES from the enkephalins was achieved by gradient elution on a µ-Bondapak C18 column (Fig. 3). Several peaks of immunoreactivity were observed in this separation but only those fractions co-eluting with authentic LES showed a marked increase in immunoreactivity after treatment with TFA or arylsulphatase (Fig. 3).

LES was also detected in the free form as part of the endogenous peptide pool in brain extracts. Material having the chromatographic properties of LES was detected by

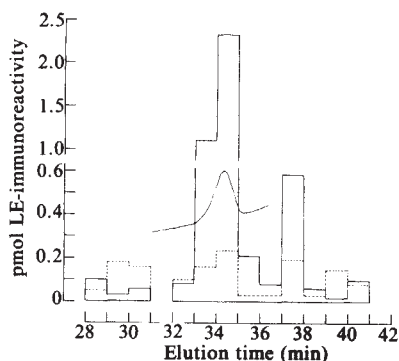


Fig. 3 Identification of the sulphate ester of Leu-enkephalin from mouse striated protein. The acidic fraction of a tryptic digest was isolated as described in Fig. 1 legend and then treated with carboxypeptidase B. The final digested sample was chromatographed on a µ-Bondapak C18 reverse-phase column (0.78 × 30 cm). The initial conditions were 5% acetic acid/propanol (90/10 v/v) with a 30-min concave gradient to give final proportions of 60/40 (v/v) and this was continued for a further 15 min. Fractions (1 ml) were collected and aliquots divided, one half being immunoassayed directly with LE1 antisera (dotted lines), and the other half treated with TFA before immunoassay (solid lines). The values correspond to 20 mg wet weight of mouse striatum. Several small peaks of Leu-enkephalin immunoreactivity were detected before TFA treatment, but only fractions 33–34, corresponding to the elution position of LES (superimposed UV absorption trace), showed a marked increase after TFA. A threefold increase in immunoreactivity was also seen with fraction 37.

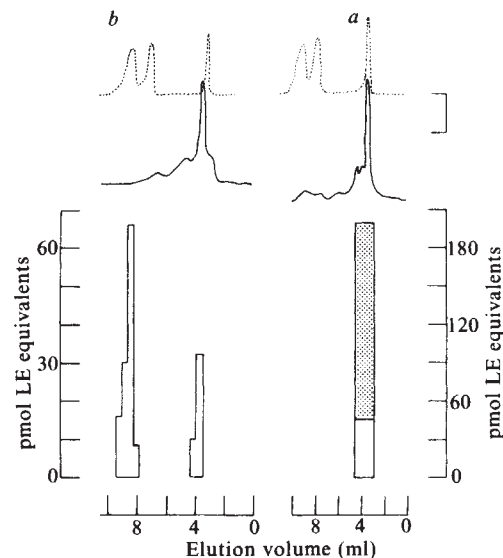


Fig. 4 Detection of endogenous acidic opioid peptides. Mouse striata were extracted as described in Fig. 1 legend and the peptide fraction separated from protein on Sephadex G-25. Acidic peptides were isolated by DEAE-Sephadex chromatography. The acidic peptides were chromatographed on a 250 × 4 mm Partisil-10-SCX column in isocratic conditions (10 mM NaH₂PO₄/H₃PO₄ pH 2.4; 0.5 and 0.4 ml min⁻¹ for traces *a* and *b* respectively). Samples equivalent to 480 mg of starting tissue were used. *a*, Elution profile of an untreated sample. *b*, Chromatogram obtained for a sample that had been incubated with TFA for 80 min. In *a*, 2 min samples were collected and divided, one half being bioassayed directly on the mouse vas deferens for naloxone-reversible activity and the other half being treated with TFA before bioassay (shaded column). One fraction in *a* contained 45 pmol of intrinsic activity; treatment of this sample with TFA increased the activity to 198 pmol. In contrast, the other fractions contained no activity either before or after TFA treatment. In *b*, 1 min fractions were collected and bioassayed directly; opiate activity was detected in a peak emerging just after the position of authentic LES and coincident with authentic Leu-enkephalin. Activity is expressed in pmol Leu-enkephalin (LE) equivalents. The upper traces are the 206 nm absorption traces for the tissue samples and the broken traces those of marker peptides. Order of elution (right to left): LES, Met- and Leu-enkephalin.

immunoassay or by bioassay after TFA treatment in several systems including silica-gel TLC, reverse-phase and cation- and anion-exchange HPLC. In Fig. 4*a*, material eluting at the position of synthetic LES was bioassayed before and after TFA treatment of the eluate fractions. Naloxone-reversible activity (~45 pmol) was detected in one fraction before TFA treatment. However, ~200 pmol of activity were detected in the same fraction after TFA treatment. As LES is only bioactive at nanomolar concentrations it was unlikely that the activity detected before TFA treatment was due to LES. Support for the existence of both LES and another intrinsically active opioid in this sample was obtained by chromatography of an identical sample on the same column after treatment with TFA. There were two peaks of activity, one (~120 pmol) corresponding to Leu-enkephalin arising from the desulphation of LES and the other, earlier peak (~40 pmol) eluting close to but slightly later than marker LES (Fig. 4*b*). The bioactivity detected in Fig. 4*a* before desulphation was quantitatively the same as that detected in Fig. 4*b* close to the LES elution point; the increase in bioactivity (Fig. 4*a*) after TFA was also quantitatively similar to the amount of Leu-enkephalin detected in Fig. 4*b*.

The intrinsically active acid opioid peptide fraction, LES, the enkephalins and more basic endogenous opioid peptides were also separated and detected by isoelectrophoresis (Table 1). We demonstrated the existence of basic opioids which migrated to the cathode (*pI* > 7.5), the enkephalins (*pI* 5.0–5.5) and a more acidic fraction (*pI* 2.9–3.1). None of the fractions showed any

Table 1 Isoelectrophoretic separation of rat brain opioid peptides

pI	Leu-enkephalin equivalent activity (pmol)	
	Untreated eluate	TFA-treated eluate
>7.5	110	120
4.9–5.5	460	425
2.9–3.2	60	48
<2.5	0	80

Three whole rat brains were homogenized in 5% TCA (10 ml per g tissue) and the solubilized peptides were absorbed on 200 mg of Poropak-Q; after a water wash the peptides were eluted with 5 ml of methanol. The methanol eluate was divided and dried. Isoelectric focusing was carried out on horizontal slab gels of Ultradex (prepared as recommended by LKB). Two runs were made with pH gradients of 5–8 and 2.5–4.0 respectively (2% Ampholines (LKB); 8 W constant power at 10 °C for 12 h). At the end of each run the gels were sliced and the peptides eluted with 2 ml distilled water. The samples were then absorbed on to Poropak-Q which was washed thoroughly with water to remove ampholines, then eluted with methanol. The dried methanol eluates were bioassayed directly on the mouse vas deferens and after TFA treatment. Trace amounts of ³H-labelled enkephalins added to the original samples were also used to establish the isoelectric points of these peptides: Leu-enkephalin = 5.4 (5.2–5.5); Met-enkephalin = 5.2 (4.9–5.3).

enhancement of activity after TFA treatment except material from the anode where LES would be expected to migrate with a pI of <2.0.

We have detected the presence of protein-incorporated LES in sheep, mouse and guinea pig striatum. The free peptide, which is also present in these species and the rat, appears to constitute 10–20% of the total free pool of Leu-enkephalin. The proportion of LES in the proenkephalin fraction may be much higher, initial studies indicating that as much as 50% of protein-incorporated Leu-enkephalin may be sulphated. We have also detected LES in the high-molecular weight proenkephalin⁴ and thus sulphation may occur early in peptide biogenesis, possibly at the nascent peptide stage. The sulphate ester of Met-enkephalin has not been detected although we cannot exclude completely its occurrence.

The significance of these findings is unknown. Sulphation may represent an inactivation mechanism, a signal for selective precursor cleavage or a means for conferring novel non-opiate activities on opioid peptides. The brain contains several endogenous enkephalin-related peptides including α -neo-endorphin⁵, dynorphin⁶, Met⁵-enkephalinyllarginyphenylalanine⁷ as well as Met- and Leu-enkephalin⁸. At present we do not know if they share common biosynthetic pathways but it will be of considerable interest to determine whether any of these other peptides undergo sulphation. In the pro-opiomelanocortin system, N-acetylation⁹ can lead to the activation of melanotropin and inactivation of β -endorphin, thus providing a flexible system for determining the nature of active products generated by a common precursor. It remains to be determined whether sulphation has a similar role in the proenkephalin system.

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Molecular size of benzodiazepine receptor in rat brain *in situ*: evidence for a functional dimer?

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Benzodiazepine tranquilizers such as diazepam and chlordiazepoxide interact with high-affinity binding sites in nervous tissue^{1,2}. The correlation between the affinities of various benzodiazepines for these sites with their clinical potencies and activity in behavioural and electrophysiological tests in animals suggests that the sites represent the functional 'receptor' whereby benzodiazepines exert their effects³. The intimate involvement of benzodiazepines with γ -aminobutyric acid (GABA) and chloride channels raised the possibility that the benzodiazepine binding site (BDZ-R) may be a protein in the GABA receptor-effector complex^{4,5}. GABA agonists enhance the affinity of BDZ-R for benzodiazepines⁶, although BDZ-R is distinct from the GABA receptor itself³. However, electrophysiological evidence suggests that the action of benzodiazepines is chloride channel, rather than receptor, directed^{7–10}. Several attempts have been made to measure the molecular weight (M_r) of BDZ-R after solubilization from brain membranes: treatment with 1% Triton X-100 followed by assay of binding activity in solute fractions separated according to molecular weight suggested¹¹ a value of ~200,000, photoaffinity labelling of BDZ-R with ³H-flunitrazepam (³H-FNZ) followed by more rigorous solubilization and gel chromatography indicated^{12,13} an apparent M_r of ~55,000 and a third approach¹⁴ a value of ~100,000. The measured molecular weight seems to depend critically on the solubilization procedure used. Chang *et al.*¹⁵ recently described the use of radiation inactivation to determine the size of BDZ-R *in situ* in calf brain membranes, and estimated a M_r of 216,000. We have also used this approach; the results reported here indicate a M_r of between 90,000 and 100,000, but this is reduced to 60,000–63,000 in membranes pretreated with GABA, suggesting the disaggregation of a normally dimeric form.

Radiation inactivation has been widely used to obtain the molecular weights of enzymes and other soluble macromolecules, and the technique has also been applied to membrane-bound proteins such as the (Na⁺ + K⁺)ATPase in erythrocytes, the tetrodotoxin binding site in neuronal membranes and the glucagon-stimulated adenylate cyclase in hepatocytes¹⁶. The method has the great attraction of allowing size determinations to be made on functionally defined membrane proteins *in situ*. High-energy particles can inactivate biological macromolecules according to classical target theory: thus, if inactivation is considered an all-or-none event, the relationship between degree of inactivation and radiation dose will be an exponential one, whose decay constant will be solely a function of molecular size. This can be computed from the dose of radiation in megarads required to reduce biological activity by a factor of 1/e (the D_{37}) using a conversion constant which has been derived both theoretically and empirically¹⁷

$$M_r = 6.4 \times 10^5 / D_{37}$$

The radiation dose delivered was independently monitored by assaying an endogenous membrane marker of known molecular weight (acetylcholinesterase); the measurements indicated a M_r of 65,000 for the enzyme, in good agreement with previously determined values¹⁷.

In the present study we irradiated *in vacuo* lyophilized neuronal membranes from rat brain with electrons accelerated through 16 MeV by the linear accelerator at Addenbrooke's Hospital, Cambridge for various times at a rate of 2 Mrad min⁻¹.

Table 1 Affinity (K_D) and capacity (B_{max}) of ^3H -FNZ binding sites determined by Scatchard analysis

	K_D (nM)	B_{max} (pmol per g protein)
Fresh membranes	2.5 ± 0.3	282 ± 11 ($n = 3$)
Lyophilized membranes	2.3	270
Irradiated membranes	2.0	90

Values are compared in freshly prepared membranes, lyophilized and reconstituted membranes, and lyophilized membranes irradiated with 10 Mrad high-energy electrons before reconstitution. Each determination used data from triplicate determinations of specific binding at eight ^3H -FNZ concentrations between 0.1 and 10 nM. For K_D values, regression analysis of the slopes of Scatchard plots revealed no significant difference between experimental groups.

The residual BDZ-R activity was measured using a conventional ^3H -FNZ radiolabelling assay and compared with non-irradiated controls. The binding properties of lyophilized membranes were not significantly different from freshly prepared membranes (Table 1); by contrast the increase in GABA receptor affinity on freezing is well documented¹⁸.

Figure 1 demonstrates that electron bombardment inactivated BDZ-R and the linear nature of the logarithmic transform suggests loss of a single component of homogeneous size; further experiments show that this linearity is preserved up to doses inactivating >90% of BDZ-R activity. Irradiation produced no change in the nonspecific ^3H -FNZ binding. Scat-

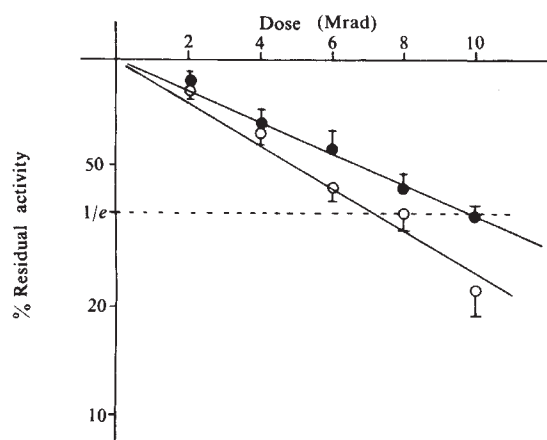


Fig. 1 Inactivation of ^3H -FNZ binding sites in rat neuronal membranes by high-energy electrons in the absence (○) and presence (●) of GABA. Membranes were prepared by homogenization of whole brain tissue 1:40 (w/v) in 50 mM Tris-citrate buffer pH 7.1, followed by 20 min centrifugation at 50,000g. The pellet obtained was resuspended and centrifuged a further four times before use. Pellets were suspended in distilled water with or without 10^{-4}M GABA and aliquots lyophilized in 5 ml glass centrifuge tubes. The tubes were evacuated and irradiated at 2 Mrad min^{-1} ; during irradiation they were cooled by a stream of air that had passed through dry ice to maintain the temperature below 60°C ; this heating had no effect on the binding properties. Following irradiation, the samples were suspended in Tris-citrate buffer at a concentration of $\sim 3\text{ mg protein per ml}$, and the amount of ^3H -FNZ binding assessed: 500- μl aliquots were incubated at 4°C for 30 min with $0.5\text{ nM } ^3\text{H}$ -FNZ and the binding terminated by filtration. 'Specific' binding was defined as the component displaceable by excess ($2\text{ }\mu\text{M}$) FNZ, and represents $\sim 95\%$ of total binding in control samples. Each sample was assayed in triplicate, and the data represent mean $\pm$ s.e.m. of six independent experiments. Residual ^3H -FNZ binding is expressed as the percentage of that seen in non-irradiated controls. In these controls, specific binding represented $18.8 \pm 0.7\text{ pmol per g protein}$, which was significantly increased ($P < 0.05$) in the presence of 10^{-4}M GABA to $22.4 \pm 1.5\text{ pmol per g protein}$. The data were plotted as a semi-log transform, and the exponential decay lines of best fit computed by linear regression analysis. Intercept values ($1/e$) were 7.2 ($5.8\text{--}8.6$; 95% confidence limits) Mrad and 10.1 ($9.1\text{--}11.1$) Mrad, without and with GABA respectively. The dose of irradiation was monitored by measuring current flow to Earth from a Faraday cup before which the samples were mounted. The current flux was regularly calibrated by reference to absorbance change in clear Perspex HX mounted in place of the samples²². The dosimetry was independently confirmed by monitoring the acetylcholinesterase activity in aliquots of the brain membranes by the colorimetric method of Ellman *et al.*²³.

chard analysis shows that loss of activity reflected a loss of binding sites rather than a change in binding characteristics—as might be expected if the inactivation were not all-or-none (Table 1). The radiation-sensitive M_r calculated from these data is $89,000 \pm 15,000$.

We also studied the molecular size of the BDZ-R in membranes treated before lyophilization with 10^{-4}M GABA, a concentration sufficient to cause a maximum increase in BDZ-R binding affinity⁶. In these membranes, the radiation-sensitive size was significantly ($P < 0.05$) decreased to $63,000 \pm 7,000$. Values obtained from a further series of five experiments were 102,000 and 60,000 without and with GABA respectively; these values are not significantly different from the original values. The simplest explanation of this effect of GABA is that BDZ-R may exist as a dimer whose disaggregation is triggered by GABA receptor activation. If, as discussed above, BDZ-R is related to the chloride ion channel, then it is possible that GABA receptor activation causes the separation of two trans-membrane proteins, thus opening a chloride ion channel; the open channel (BDZ-R monomer) would have a higher affinity for the benzodiazepines than the closed channel (BDZ-R dimer). A similar model of protein transformation in a receptor-ionophore complex has been postulated for GABA on physicochemical grounds¹⁹, and parallels current models for the nicotinic acetylcholine receptor²⁰.

The apparent molecular size reported here for BDZ-R, between 90,000 and 100,000, is not significantly different from the value obtained by Braestrup *et al.*¹⁴. The more rigorous purification of photoaffinity-labelled BDZ-R which yields a weight of 55,000 may produce the 'monomeric' BDZ-R we have postulated above. The 200,000- M_r moiety labelled after solubilization¹¹ is still sensitive to GABA²¹ and co-chromatographs with ^3H -labelled receptors¹¹, thus may represent the GABA receptor-ionophore complex, rather than independent BDZ-R. It is not clear why Chang *et al.*¹⁵ found a value of $\sim 200,000$, using a similar radiation inactivation technique. However, they used a different species (ox) and treated the brain membranes with Triton X-100, which may affect the state of aggregation of membrane proteins.

The present study demonstrates the feasibility of using radiation inactivation not only to measure the size of membrane-bound receptors, but also to analyse physicochemically the effects of interactions between transmitters, receptors and effectors on their molecular properties.

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Use of carboxyfluorescein diacetate to study formation of permeable channels between mouse blastomeres

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The transmission of signals between cells of the early preimplantation embryo may be important in the recognition of relative cell position, thereby influencing the direction of cell differentiation¹⁻⁵. Gap junctional (water-permeable) channels may provide one route for such signal transmission⁶. A previous report⁷, in which intact, zona-free mouse embryos were impaled by microelectrodes, demonstrated that gap junction-mediated dye transfer and electrical continuity were absent between blastomeres of two- and four-cell embryos but appeared during the eight-cell stage. It is at the eight-cell stage that compaction occurs, during which cells polarize³, flatten on each other^{3,8} and show the first morphological evidence of intercellular junctions^{9,10}. We report here the use of a simple, non-iontophoretic method to detect the passage of a low-molecular-weight dye between aggregated blastomeres of the early preimplantation mouse embryo. Cells are incubated in the nonfluorescent, non-polar reagent, 6-carboxyfluorescein diacetate (CFDA)^{11,12}, which enters the cell freely, where it becomes entrapped following enzymatic conversion¹³ to the hydrophilic fluorophore 6-carboxyfluorescein (CF; molecular weight 370). The labelled cells may then be aggregated with unlabelled cells and the formation of communicating channels between cells is revealed by the passage of dye from labelled to unlabelled blastomeres. CFDA provides better results than previously used fluorescein esters^{14,15}, which hydrolyse to a much less hydrophilic fluorophore that leaks from the cell relatively quickly^{11,15}. Using CFDA we have confirmed that the capacity to form channels is lacking at all stages from the mature oocyte to the four-cell embryo but appears during the first 6 h of the eight-cell stage, preceding the fully compacted state.

In preliminary experiments, it was established that CFDA (derived from a 10 mg ml⁻¹ stock solution in acetone) at concentrations of 4–40 µg ml⁻¹ in phosphate-buffered medium (PB1)¹⁶ labelled cells brightly, with no detectable effects on viability or subsequent division. The dye was distributed evenly throughout the cytoplasm (Fig. 1), leaked only slowly from two-, four- or eight-cell blastomeres, as reported for the lymphocyte¹¹, although somewhat faster from unfertilized or fertilized eggs, and was not taken up in detectable quantity by unlabelled blastomeres co-cultured with labelled blastomeres. Free transfer of dye was detected only between aggregated blastomeres from the eight-cell embryo (Figs 1, 2).

The first sign of dye transmission was the appearance of a weak fluorescence in the unlabelled blastomeres, which subsequently increased, concomitant with a decline in the fluorescence of the labelled blastomeres, until all blastomeres fluoresced with similar intensity (Fig. 1). No dye was transferred between aggregated pairs of unfertilized eggs (Fig. 3a, b; 43 pairs observed at 15–17 h following injection of human chorionic gonadotropin [hCG]), consistent with earlier studies¹⁷, or between one-cell embryos (18 pairs observed at 20 h post-hCG). Blastomeres from two- and four-cell embryos were not able to transmit dye (68 and 20 pairs examined respectively), even when cultured for up to 5 h (Figs 2, 3). A similar result was obtained from all pairings between cells of different embryonic stages from mature oocyte to eight-cell embryo (229 aggregates examined in various combinations), even when one cell of the aggregated pair was from a population proved capable of transmitting dye within itself (Fig. 3).

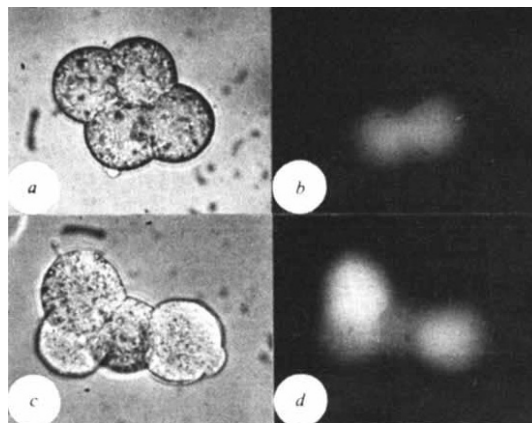


Fig. 1 Passage of CF between blastomeres derived from eight-cell mouse embryos. Female CFLP mice (Hacking and Churchill) were superovulated by intraperitoneal injection of 5 IU (international units) pregnant mare serum (PMS; Folligon) and 5 IU human chorionic gonadotropin (hCG) (Chorulon) 44–48 h later. Mice were mated, and compacted eight-cell embryos (~70 h post-hCG) were recovered, their zonae pellucidae removed and they were then disaggregated to either single cells (1/8) or pairs of cells, according to established procedures³. Blastomeres were then taken through four changes of CFDA (4 µg ml⁻¹) in phosphate-buffered medium (PB1)¹⁶ without protein, and incubated in suspension in the final change of labelling medium for 2 min and then rinsed three times in PB1 containing 4 mg ml⁻¹ bovine serum albumin (BSA). Labelled cells were then aggregated to unlabelled cells using phytohaemagglutinin (Gibco Biocult) (5% v/v of stock solution in Ca²⁺-deficient medium 16 + 6 mg ml⁻¹ BSA), shown previously to have no obvious effect on development^{3,19}, rinsed in PB1 + BSA and cultured as described previously³ in drops of medium 16 + 4 mg ml⁻¹ BSA at 37 °C in 5% CO₂ in air, under oil in plastic cell culture dishes (Sterilin). After 3 h, the aggregates were mounted in PB1 + BSA on a tissue typing plate (Baird and Tatlock) and viewed with a Zeiss photomicroscope (filter set 48 77 09 with incident source HBO 200 for epifluorescence [b, d] and transmission optics for brightfield [a, c]). Photographs were taken on Kodak Tri-X 35 mm film. Fluorescence exposures were of 2 min duration. a, b, No transfer of CF between a labelled and unlabelled pair of blastomeres has yet occurred. c, d, Complete exchange of dye has occurred between the labelled pair and the unlabelled pair. Scale bar, 10 µm.

To measure the time taken to establish water-permeable channels between cells with an existing capability to transfer, individual blastomeres from compacted, late eight-cell embryos were labelled and aggregated with similar, unlabelled cells. Couplets were analysed at various times after aggregation for evidence of dye transmission (Fig. 2, left-hand curve). Four hours elapsed before all the pairs showed complete dye transmission, although this had occurred in some pairs within the first hour. Whether these early transmitting pairs resulted from assembly of pre-existing proteins or the synthesis *de novo* of gap-junctional proteins is not known, although in Cl-1d cells the minimum time required for synthesis and construction of permeable channels has been estimated to be 4–6 h (ref. 18).

To determine the time at which an eight-cell blastomere first develops the capacity to transmit dye, labelled 2/8 pairs were aggregated at a known time after their formation by division of 1/4 blastomeres, with unlabelled 2/8 pairs of the same age, and cultured for 3 h before analysis. The results are shown in the right-hand curve of Fig. 2; the slope of the curve is similar to that on the left, and presumably reflects the variability in time required to assemble functional junctions and transmit dye. However, the whole curve is shifted to the right by 2–3 h; it seems that ~2–3 h must elapse before a newly formed 1/8 blastomere becomes competent to assemble channels and transmit the dye. Junction formation, therefore, is a relatively early event in the life of the eight-cell embryo, coinciding with the induction of polarity¹⁹ and preceding cell flattening³.

This conclusion is further supported by experiments in which complete eight-cell embryos were aggregated and assayed for transfer of CF (Fig. 4). Transmission of dye occurred when both embryos were in a precompact state at the time of aggregation

(Fig. 4a, b), suggesting that the competence to form water-permeable channels must precede complete cell flattening. However, when both embryos were compacted at aggregation, no transfer occurred (Fig. 4c, d), despite the proven ability of individual blastomeres isolated from compacted embryos to transmit the dye (Fig. 4e, f). Presumably, in compact embryos, the completely microvillous outer surfaces²⁰⁻²² of the cells either lacked gap-junctional proteins or were unable to assemble them in the time available to form functional channels.

The use of CFDA provides a simple technique for studying gap-junctional communication between small groups of cells. Moreover, many observations can be made within a short period. The results reported here confirm and extend previous

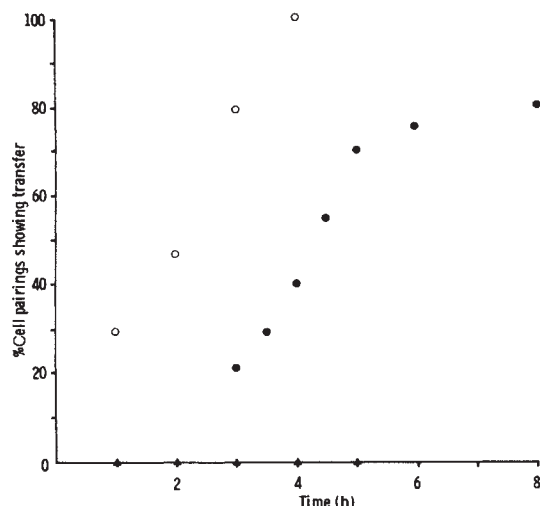


Fig. 2 Capacity of blastomeres to transmit CF. ○, Compact eight-cell embryos were disaggregated to single cells, some of which were labelled with CF and reaggregated with unlabelled cells. Couplets were examined for dye transfer at 1, 2, 3 or 4 h (67 couplets examined). For comparison, results with blastomeres derived from two-cell embryos (41 h post-hCG) and treated similarly are shown (Δ; 68 couplets examined). The horizontal axis represents time (in h) after aggregation at which aggregates were examined for dye transmission. ●, Four-cell embryos were disaggregated to 1/4 blastomeres, which were cultured *in vitro* and examined at 1/2 h intervals. Any cells dividing to 2/8 were collected³, and the couplets were cultured for 0–5 h. At 0, 0.5, 1, 1.5, 2, 3 and 5 h, some couplets were labelled and aggregated to unlabelled couplets of the same age post-division. The aggregates were then cultured for 3 h and analysed for dye transmission as described in Fig. 1 legend. Fifty-nine pairs of couplets were examined in total. The horizontal axis represents total time (in h) elapsed since division to 2/8 had occurred and includes in each case the 3 h between labelling plus aggregation and examination for dye transmission. In all cases controls were prepared consisting of co-incubated, but unpaired, labelled and unlabelled cells that had been subjected to otherwise identical experimental conditions to the paired cells. The failure of 100% of paired cells to transmit merely reflects the choice of a 3-h transfer period (see the left-hand curve in which only 80% of pairs showed transmission after 3 h).

observations using conventional iontophoretic techniques⁷. Thus the capacity to assemble functional gap-junctional channels does not develop until the early eight-cell stage when they form rapidly, due either to the insertion of gap-junctional proteins or to reorganization of existing gap-junctional proteins within the membrane²³. It will be important to establish whether gap junction formation is required for the coordination of events resulting in polarization and cell flattening and if so, whether such coordination depends on the evening out of metabolic potential²⁴ or the passage of specific signalling molecules⁶ between cells. The available evidence does not suggest that gap junctions are involved in the induction of polarity because two- and four-cell blastomeres can induce polarity in eight-cell blastomeres¹⁹, yet cannot transmit to them. It remains to be determined, however, to what degree junctions are involved in subsequent events of early mouse development.

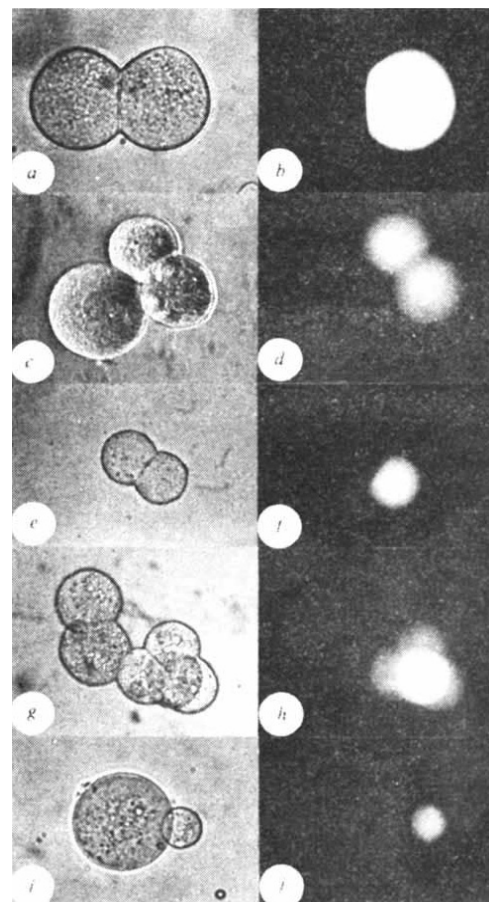


Fig. 3 Failure of CF to pass between cells taken from embryos at different stages of early development. Individual blastomeres were labelled with CF, incubated and photographed as described in Fig. 1 legend, whilst whole embryos were labelled within their zonae for 10 min, their zonae removed and then similarly treated. All aggregates were cultured for at least 3 h before examination. Phase-contrast (a, c, e, g, i) and fluorescence (b, d, f, h, j) images of the following couplets are shown: a, b, unfertilized egg; c, d, fertilized egg + two-cell embryo; e, f, 1/4 + 1/4 blastomeres; g, h, two-cell + four-cell embryos; and i, j, unfertilized egg + 1/8 blastomere. (No transfer was apparent even when the egg was labelled; thus failure to detect transfer did not result from dilution of CF.) Scale bar, 50 μm.

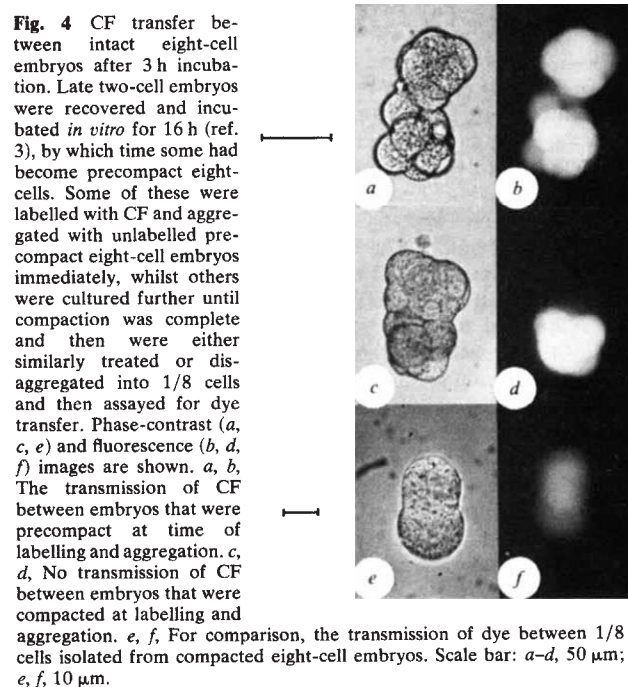


Fig. 4 CF transfer between intact eight-cell embryos after 3 h incubation. Late two-cell embryos were recovered and incubated *in vitro* for 16 h (ref. 3), by which time some had become precompact eight-cells. Some of these were labelled with CF and aggregated with unlabelled precompact eight-cell embryos immediately, whilst others were cultured further until compaction was complete and then were either similarly treated or disaggregated into 1/8 cells and then assayed for dye transfer. Phase-contrast (a, c, e) and fluorescence (b, d, f) images are shown. a, b, The transmission of CF between embryos that were precompact at time of labelling and aggregation. c, d, No transmission of CF between embryos that were compacted at labelling and aggregation. e, f, For comparison, the transmission of dye between 1/8 cells isolated from compacted eight-cell embryos. Scale bar: a–d, 50 μm; e, f, 10 μm.

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Crystal structure of a polyfunctional macrocyclic K^+ complex provides a solid-state model of a K^+ channel

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Molecular channels are thought to be important in ion exchange processes across membranes, and biophysical and physiological data point to the existence of ion-specific Na^+ and K^+ pores (see, for example, refs 1, 2). The nature and structure of these entities, as well as the molecular mechanism of ion flow through channels, are still largely unknown, whereas cation transport via facilitated diffusion by natural or synthetic carrier molecules has been extensively investigated in both natural and model membrane systems^{3–5}. The formation of transmembrane cation channels has been studied mainly with linear peptides (gramicidin A, alamethicin; refs 3, 7, and refs 6, 8 and refs therein). The cylindrical macrotricyclic molecules are suitable model species for investigating both the synthetic construction of molecular channel subunits and their cation binding properties as they form cation inclusion complexes of cryptate type and undergo intramolecular cation jump processes^{9–11}. We report here the crystal structure of the potassium complex (1, 1.5 KBr, 3.5 H_2O) of the chiral macrocyclic tetracarboxamide 1, a member of a group of functionalized macrocyclic polyethers^{12,13} which strongly bind alkali and ammonium cations¹⁴. This structure presents a solid-state model of a molecular channel, in which the macrocyclic units are organized in a polymolecular stack and the K^+ cations are located alternatively inside and on top of successive macrocycles, as in a frozen picture of potassium ion propagation through the stack.

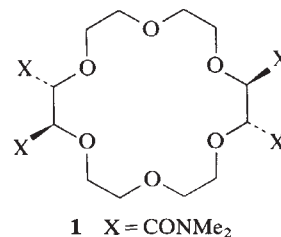


Figure 1 shows the molecular crystal packing of the above complex along the [110] axis. The structure contains a polymolecular organic unit resulting from the repetition of a stacked dimeric asymmetric unit $[(1, K)_2(H_2O)_3]^{2+}$, and is stabilized by hydrogen bonding of the water molecules with each other ($O \cdots O$ length 2.63 Å) and with six amide carbonyl groups ($O \cdots O$ length 2.50–2.95 Å), as well as by one ion dipole interaction ($K^+ \cdots O$ 2.83 Å). The counterions are located in a polymeric chain $[KBr_3(H_2O)_4]^{2-}$ parallel to the organic one, made of alternating water-bridged KBr and BrBr fragments ($Br \cdots O$ 3.3–3.45 Å). The stability of the crystal packing is completed by $Br \cdots CH_3$ van der Waals' interactions (3.68–3.76 Å) between the cationic and anionic chains.

The organic part of this structure has many attractive features. (1) The solid-state stacking of the macrocyclic units builds up an intermolecular arrangement clearly suggestive of a channel structure. The mean planes of the macrocycles are parallel to

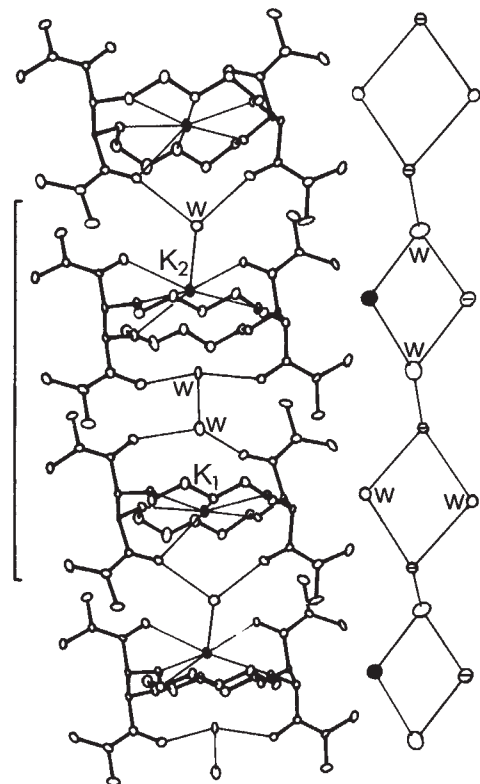


Fig. 1 Molecular packing of the complex $[(1, K)_2, 3H_2O]^{2+} \cdot [KBr_3, 4H_2O]^{2-}$ down the [110] vector. Intermolecular contacts between the oxygen atoms of water molecules (W), and K^+ (●) or Br^- (○) ions are visualized by thin lines. Crystals belonging to the triclinic space group P1 were obtained by slow evaporation of an equimolecular solution of 1 and KBr in $CHCl_3/MeOH/H_2O$ ($a = 10.73$ Å, $b = 11.27$ Å, $c = 15.78$ Å; $\alpha = 109.85^\circ$, $\beta = 99.35^\circ$, $\gamma = 92.18^\circ$). The crystal used had a size of $(0.4)^3$ mm³. 6669 significant ($I > \sigma(I)$) reflections were collected up to $2\theta = 140^\circ$ on an automated Nonius CAD4 diffractometer, using nickel-filtered Cu K α radiation. The structure was solved by the standard heavy atom method followed by Fourier syntheses; final refinement by full-matrix least squares with anisotropic temperature factors for all non-hydrogen atoms led to an R factor of 0.096 for 799 variables. Atomic coordinates are available on request and will be deposited in the Cambridge Crystallographic Data Centre.

each other and two neighbouring units are slightly shifted in a staircase-like fashion, due to steric hindrance between the lateral appendages.

(2) Both the macrocycles composing the asymmetric unit are in a relaxed conformation, similar to that found in other 18-crown-6-type complexes^{15,16}.

(3) The side chains $-\text{CON}(\text{CH}_3)_2$ are in an axial conformation, on both sides of the macrocyclic ring and direct their polar amide carbonyl groups into the internal space of the ligand.

(4) As a result of features (2) and (3) all apolar groups (methyl and methylene) of the macrocyclic units are on the outside. The previously determined structures of related compounds having secondary amide residues as lateral branches ($\text{X} = \text{CONHR}$ in 1)¹⁵ had already shown a *trans*-dial arrangement of the branches, but the carbonyl groups were directed outwards, with formation of an intramolecular $\text{N}-\text{H}\cdots\text{O}$ hydrogen bond between the CONH residues and the nearest ring ether oxygen atom. Consequently, the simple interconversion between secondary and tertiary amide functions allows a conformational control of the orientation of the polar groups towards the exterior or the interior of the structure respectively. This feature is of considerable interest for designing receptor molecules built on the basic macrocyclic unit 1.

(5) There are two distinct K^+ -binding schemes. In one complex of the asymmetric unit, the cation (K_1^+ , Fig. 1) is located inside the macrocyclic ring, 0.25 Å from its mean plane; the receptor site includes all six ether oxygen atoms ($\text{K}_1^+\cdots\text{O}$ length 2.80 Å; $\sigma = 0.05$) and one carbonyl group ($\text{K}_1^+\cdots\text{O}=\text{C}$ length 2.76 Å). The other potassium ion (K_2^+ , Fig. 1) is located on top of the macrocycle, at 1.13 Å from its mean plane; it is bound to only three ring oxygen atoms ($\text{K}_2^+\cdots\text{O}$ length 2.85 Å; $\sigma = 0.06$), to two amide carbonyl groups ($\text{K}_2^+\cdots\text{O}=\text{C}$ lengths 2.69, 2.89 Å) and to the oxygen of a water molecule ($\text{K}_2^+\cdots\text{O}$ 2.83 Å). These two modes of K^+ binding may be considered as two steps in a hopping mechanism of cation flow through the pore-like structure. Enniatins form sandwich complexes¹⁷ like the RbNCS complex of enniatin B, in which every Rb^+ ion is bound to carbonyl oxygens of two ligand molecules¹⁸.

(6) The polar interior of this solid-state channel contains three water molecules per asymmetric unit. They solvate the K^+ and amide groups, and provide H-bonding links between successive macrocyclic units.

In conclusion, the present structure possesses three main features which are expected to have a role in ion flow through membrane protein channels: a chain of cation binding units; polar inside/apolar outside orientation of residues; and mixed-site cation binding. It may therefore be considered a solid-state model of a molecular channel with cation propagation through the channel from one binding site to the next. The stepwise connection of macrocyclic units (Fig. 1) by their lateral appendages would allow the construction of a true artificial molecular channel, whose properties depend on the choice of the basic unit and of the linking branches. Synthetic work along these lines is in progress.

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Transient nature of early haematopoietic spleen colonies

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Certain cells (CFU-S) in the haematopoietic tissues of mice are able to form macroscopic nodules in the spleen 7–14 days after injection in heavily irradiated recipient mice¹. At 7–9 days, most of the spleen colonies contain recognizable cells of one predominant haematopoietic lineage², and do not contain cells capable of spleen colony formation on injection in secondary hosts³. However, by 14 days most of the spleen colonies do contain cells of more than one line of haematopoietic differentiation², as well as precursor cells capable of again generating similar multilineal spleen colonies on retransplantation³. These observations have led to the widely accepted conclusion that most spleen colonies are derived from pluripotent 'stem' cells³, and to the suggestion that the apparent transformation in character of spleen colonies from unilineal at early times to mixed later on reflects the local operation of 'instructive' differentiative signals in the splenic environment². The reasoning behind these suggestions implicitly assumed that late colonies simply represented a further stage in the development of the same colonies observable at earlier times. Here, we present direct evidence against this assumption. Our data indicate that most spleen colonies identified as surface nodules at days 7–8 are neither multipotential nor self-maintaining, but rather are destined to disappear from the spleen within 72 h. The observations set limits on the validity of the spleen colony method as an assay for self-maintaining pluripotent progenitor cells, and give cause for reassessment of the data on which widely accepted views concerning the regulation of such cells are based.

Initial experiments compared individual spleen colonies at early and late times in their development in terms of their content of cells capable of growth in culture. Such data would provide two kinds of information concerning the character of a spleen colony. First, the presence or absence of 'primitive' haematopoietic precursors capable of extended proliferation in culture would indicate the potential of the spleen colony for further growth. Second, the number of different kinds of cells growing in the cultures would supply information on the differentiative potential of the original spleen colony-forming cell.

Adult mouse bone marrow cells were injected into heavily γ -irradiated syngeneic recipients, and after varying time intervals, the recipient spleens were excised and examined at $\times 11$ magnification for surface colonies. A total of 156 consecutively identified colonies were dissected out from spleens containing 9 or fewer surface nodules. Particular care was taken to exclude the occasional colonies whose geometry suggested possible confluence of more than one colony. Single cell suspensions were prepared from each colony. Most of the cells were plated in methylcellulose cultures to assay for haematopoietic precursor cells, and the remaining cells were centrifuged onto glass slides for morphological evaluation. From the culture results, spleen colonies were classified as P^+ or P^- according to whether or not they contained any relatively 'primitive' precursor cells, defined as cells capable of forming mixed myeloid/erythroid clones in culture containing at least 1,000 cells, or clones containing at least 50 mononuclear or granulocytic cells, or at least 200 erythroid cells. Almost all spleen colonies which contained *in vitro* clonable mononuclear or granulocytic precursors also contained erythroid precursors. The proportion of each colony plated was such that the absence of growth in culture signified

the presence within the spleen colony of fewer than eight clonable precursors in the case of large spleen colonies, and fewer than one or two in the case of spleen colonies consisting of $\leq 200,000$ cells.

Figure 1a shows the results for 7–8-day spleen colonies. Most colonies consisted predominantly of recognizable erythroblasts and contained no 'primitive' precursors. Although devoid of primitive cells clonable *in vitro*, these P^- colonies did contain high numbers (up to 25% of the cells) of relatively mature erythroid precursors (CFU-E) capable of forming small clones of 8–60 erythroblasts in culture. Few spleen colonies contained $>20\%$ of morphologically unrecognizable cells. The frequency

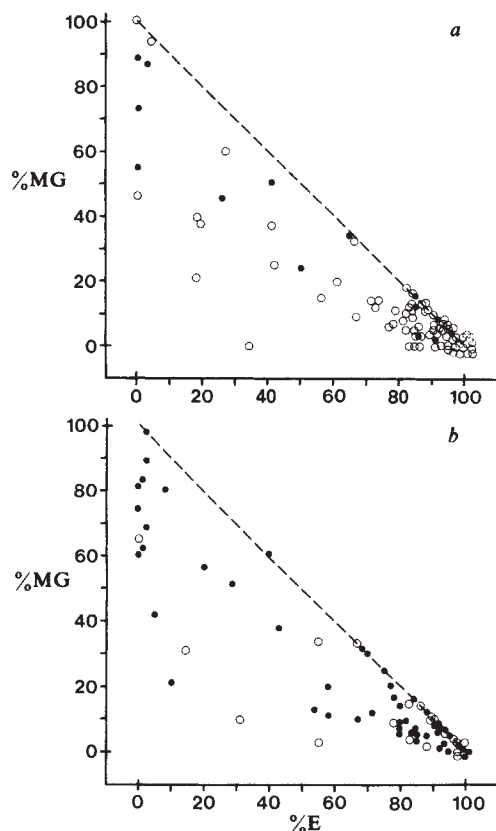


Fig. 1 Distribution of spleen colonies according to the character of their constituent cells. Each point represents one spleen colony, indicating presence (●) or absence (○) of primitive precursors capable of proliferating in culture, and the percentage of the cells identifiable morphologically as erythroid (E) or mononuclear/granulocytic (MG). *a*, Composition of spleen colonies after 7–8 days of growth in the spleen; *b*, after 9–12 days. Total numbers of spleen colonies examined were 37, 49, 14, 30, 17 and 9 on days 7–12, respectively. Female 8–12-week old BALB/c mice were γ -irradiated (800 R, ^{137}Cs) and injected intravenously with 1 or 3×10^4 nucleated syngeneic bone marrow cells suspended in IMDM⁸ with 5% fetal calf serum (FCS). Controls injected with cell-free suspending medium had <1 colony per 20 spleens. Spleen colonies were dissected out and gently dispersed into single cell suspensions in IMDM containing 50% FCS. Some of the cells were cytocentrifuged onto glass slides, fixed in methanol and stained with Gurr's Giemsa. Cells were assigned to erythroid, mononuclear, granulocytic or megakaryocytic lineages on the basis of nuclear size, form, degree of condensation and presence of nucleoli, nuclear/cytoplasmic ratio, and cytoplasmic basophilia and granulation, according to classical morphological criteria. Cells were cultured at a maximum density of 10^5 per ml in IMDM containing α -thioglycerol, methylcellulose, bovine serum albumin, 15% FCS, iron-saturated transferrin, lecithin, oleic acid, cholesterol, 1 IU ml^{-1} human urinary erythropoietin (all as detailed elsewhere⁸) and 10% conditioned medium from WEHI-3 cells⁹ as a source of erythroid burst-promoting activity⁵ and of granulocyte/macrophage colony-stimulating activity. Small erythroid colonies from CFU-E were counted after 2 days of culture, and larger erythroid or mixed myeloid/erythroid bursts and granulocyte/macrophage colonies were counted on days 7, 10 and 14.

of such cells correlated neither with the predominant morphological type nor with the presence of primitive precursors.

Figure 1b shows pooled results from spleen colonies analysed at later times of development (days 9–12). Three-quarters of the colonies now contained primitive precursors, a proportion consistent with earlier observations³ on CFU-S in later spleen colonies but higher than that reported by Gregory and Henkelman in 11–12-day spleen colonies⁴, possibly because of the addition to our cultures of a controlled concentration of 'burst-promoting activity'⁵. Half of our P^+ colonies showed mixed differentiation morphologically. Most P^- colonies again consisted predominantly of recognizable erythroblasts.

The relationship between early and late spleen colonies was then examined directly as follows. On days 7 or 8 after irradiation and marrow injection, recipient mice were anaesthetized and a small abdominal incision was made over the spleen. Each spleen was carefully inspected at $\times 11$ magnification without excision and a topographical sketch made. The incision was then closed and splenic colony development permitted to continue for an additional 2 to 5 days before the mice were killed, and their spleens excised and again mapped topographically. Control experiments using irradiated mice which had not received bone marrow indicated that the surgical procedure did not itself induce the appearance of endogenous spleen colonies. The accuracy of the topographical sketches was confirmed in most cases by direct photography of the spleens. Figure 2 shows a typical result. Two of five colonies present on the spleen at day 8 are no longer visible at day 10, while, conversely, two of five colonies visible on day 10 were not visible at day 8. The aggregate results of the mapping experiments are presented in Table 1. Of the surface colonies visible on days 7 or 8, only half were still visible on day 10, whereas only one-third of colonies visible on day 10 had visible antecedents on days 7 or 8. In a separate experiment on a smaller scale, 80% of colonies visible at day 7 had vanished by day 12.

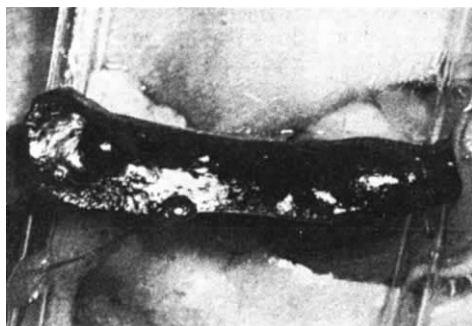
These results show spleen colonies to be divisible into two major categories. The first colony type (P^-) grows rapidly to achieve macroscopic dimensions at the spleen surface within 7–8 days and typically consists almost entirely of erythroid cells. Devoid of primitive cells capable of sustaining their growth, the P^- colonies soon vanish from the spleen, presumably as their cells differentiate terminally into erythrocytes and enter the circulation. A second colony type (P^+) grows more slowly in the first week, and at 7 days few can be identified grossly at the spleen surface. Because their growth is sustained well beyond day 9 by the continuous presence of primitive cells, these P^+ colonies gradually become the predominant colony type as the P^- colonies recede and disappear.

These findings provide a fresh context for interpreting the significance of the 'transient endogenous erythroid spleen colonies' reported earlier^{6,10,11}. Our observations and interpretations are also consistent with those of Hodgson and Bradley⁷ on late-appearing spleen colony formation by fluorouracil-resistant precursors. Our results imply that the spleen colony method provides a measure of pluripotential cells

Table 1 Number of surface spleen colonies visible at $\times 11$ magnification

	Days 7–8	Day 10
Transient	51	0
Persistent	50	50
Delayed in appearance	0	97
Total	101	147
	Day 7	Day 12
Transient	19	0
Persistent	5	5
Delayed in appearance	0	44
Total	24	49

Fig. 2 The same spleen photographed after 8 (left) and 10 (right) days of spleen colony development. Drawings indicate the locations of the colonies. Colonies marked ∇ disappeared in this time interval; those marked $\blacktriangledown$ were delayed in appearance.



capable of extensive proliferation only where macroscopic colonies are scored at 11 days or later. When spleen colonies are scored earlier than this, as has been done in almost the entire literature on the subject, the method appears to detect a population of cells more closely akin to the cells (BFU-E) which form large erythroid colonies after a similar length of time in semi-solid tissue culture^{4,5}. Finally, our observations show that late multilineal P⁺ spleen colonies are not derived from the unilineal P⁻ colonies visible at earlier times, and therefore remove a key supporting element from earlier arguments for 'instructive' local

environmental control of self-renewal and determination at the level of multipotential progenitors.

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Isolation of membrane components associated with human red cell antigens Rh(D), ($\bar{c}$), (E) and Fy^a

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The isolation of human red cell membrane components associated with Rhesus (Rh) and Duffy (Fy) blood group antigen expression has been largely frustrated due to the small quantities in the membrane¹⁻⁵ and their probable integral membrane nature requiring lipid for optimum antigen expression⁶⁻¹⁰. The disadvantage of reported immunoaffinity techniques for investigating Rh(D) has been non-specific adsorption which complicates identification of antigen-associated components^{11,12}. Thus, Rh(D) has been attributed to a 7,000 molecular weight (MW) fraction¹¹ and a subpopulation of band 3 (ref. 13; MW ~90,000)¹². We report here a technique, with negligible non-specific adsorption, for the isolation of blood group antigen-antibody complexes and their analysis by SDS-polyacrylamide gel electrophoresis (PAGE). Radiolabelling techniques facilitated identification of antigen-associated membrane components. We found that expression of Rh(D), ($\bar{c}$) and (E) was associated with a component(s) of 28,500 apparent MW, with additional 50,000- and 68,000-MW components associated with Rh(D). In contrast, Fy^a is associated with 39,500- (possibly PAS 2), 64,000- and 88,000-MW components, the latter having a similar mobility in SDS-PAGE to band 3.

The demonstration of stable Rh(D)-¹²⁵I-anti-Rh(D) complexes in detergent solution¹⁰ together with the known affinity of protein A for the Fc region of IgG¹⁴ suggested a rapid and generally applicable technique for the isolation of detergent-solubilized immune complexes of blood group antigen with the corresponding IgG antibody by adsorption to protein A-Sepharose (Pharmacia). The observation¹⁰ that the optimum expression of Rh(D) occurs only in the native membrane environment suggested that it was advisable to form immune complexes by incubating antibody with the intact red cell.

¹²⁵I surface-labelled red cells were incubated (sensitized) with an appropriate IgG antibody and, following solubilization of the membrane in non-ionic detergent, the immune complexes isolated by adsorption to protein A-Sepharose. The complexes were eluted with SDS and analysed, in reducing conditions, by SDS-PAGE. The presence of ¹²⁵I label which indicated membrane components exposed on the outer surface of the red cell, a prerequisite for implication in expression of blood group antigen activity, was detected by autoradiography of the dried SDS-PAGE gels.

The total radioactivity present in complexes isolated from cells sensitized with anti-Rh(D), anti-Rh($\bar{c}$), anti-Rh(E) and anti-Fy^a antibodies is shown in Table 1. When compared with the negative controls, all complexes contained significant levels of ¹²⁵I, indicating the presence of surface exposed membrane components. The results obtained with anti-Rh(D) show, in particular, the effect of antigen dosage. The rare cells of genotype -D-/-D- ('super D') have ~2.5-5 times the amount of surface-exposed Rh(D) antigen compared with cells homozygous for Rh(D), such as $\bar{c}DE/\bar{c}DE$ (R₂R₂) cells³. The increased levels of ¹²⁵I in complexes from super D cells is consistent with this observation and is evidence for the specific association of the labelled component with Rh(D) antigen.

Table 1 Adsorption, using protein A-Sepharose, of immune complexes present in Triton X-100 extracts of ghosts prepared from ^{125}I -labelled human erythrocytes sensitized with blood group antibody IgG and their elution with SDS

Cell genotype	Antibody specificity used*	Immune complex on protein A-Sepharose (1) (c.p.m.)	Protein A-Sepharose residue (post SDS elution) (2) (c.p.m.)	SDS eluate of protein A-Sepharose (1-2) (c.p.m.)
-D/-D- (super D)	anti-Rh(D) (1:1,028, 10 ml)	211,490	8,402	203,088 (n)
$\bar{c}DE/\bar{c}DE$ (R_2R_2)	anti-Rh(D) (1:1,028, 10 ml)	72,298	4,989	67,309 (a)
$\bar{c}de/\bar{c}de$ (rr)	anti-Rh(D) (1:1,028, 10 ml)	4,978	2,202	2,776 (b)
$\bar{c}DE/\bar{c}DE$	anti-Rh($\bar{c}$) (1:512, 25 ml)	26,919	1,634	25,285 (c)
CDe/CDe (R_1R_1)	anti-Rh($\bar{c}$) (1:512, 25 ml)	4,189	1,700	2,489 (d)
$\bar{c}DE/\bar{c}DE$	anti-Rh(E) (1:128, 25 ml)	23,656	1,604	22,052 (e)
CDe/CDe	anti-Rh(E) (1:128, 25 ml)	5,083	571	4,512 (f)
$Fy(a+b-)^{\dagger}$	anti-Fy ^a (1:128, 25 ml)	23,912	1,530	22,382 (g)
$Fy(a-b+)^{\dagger}$	anti-Fy ^a (1:128, 25 ml)	4,060	1,258	2,802 (h)

Washed human erythrocytes were surface-labelled with ^{125}I using the procedure of Reichstein and Blostein¹⁸; packed cells (1 ml) were incubated (37 °C, 90 min) with 20 ml of incubation buffer and washed (2 × 20 ml) with phosphate-buffered saline (PBS), pH 7.4. Packed cells were incubated (37 °C, 15 min) with 0.7 ml PBS, lactoperoxidase (EC1.11.1.7; Calbiochem grade B; 13 μl , 2.5 mg ml^{-1} in PBS), β -D-glucose oxidase (EC1.1.3.4; Sigma type V; 40 μl of 1:100 dilution in PBS), Na ^{125}I (100 mCi ml^{-1} ; Amersham; 10 μl (100 μCi) of 1:10 dilution in 0.01 M NaOH) and α -D-glucose (0.22 ml, 18 mg ml^{-1} in PBS prepared 24 h before use to allow mutarotation to occur), and washed with PBS (4 × 20 ml). The use of fresh batches of ^{125}I was critical to the success of the procedure. Old batches of ^{125}I did not significantly label Rhesus antigen-associated components, possibly due to oxidation of iodide by admitted air during storage. Packed, labelled (~30 μCi per ml packed cells) cells were incubated (37 °C, 60 min) with blood group antibody (whole human serum containing IgG blood group antibody of known specificity was obtained from volunteer donors and the titre determined by manual indirect antiglobulin test¹⁹), washed with PBS (3 × 20 ml) and the ghosts isolated²² (1 ml of packed cells plus 40 ml of 5 mM NaH $_2$ PO $_4$ /Na $_2$ HPO $_4$, pH 7.4 (4 °C), mix, centrifuge (48,000g, 15 min, 4 °C) repeat wash). Ghosts were incubated (15 min, room temperature) with 6 ml of PBS-Triton (PBS containing 1% (w/v) Triton X-100; BDH, scintillation grade), centrifuged (48,000g, 15 min, 4 °C) and the supernatant carefully aspirated. Immune complexes were adsorbed from the PBS-Triton extract (contained in a 10 ml, conical, plastic centrifuge tube) with 0.5 ml of a 10% (v/v) suspension of protein A-Sepharose in PBS-Triton and the mixture incubated (30 min, room temperature) with continuous roller mixing. The protein A-Sepharose beads were washed (4 × 10 ml) with PBS-Triton, counted for ^{125}I , washed with distilled H $_2$ O (10 ml) and the packed beads resuspended in 1% SDS (1 ml) and incubated at 37 °C for 30 min. The supernatant was lyophilized by vacuum centrifugation²³ and reconstituted in 0.15 ml of electrophoresis sample buffer (0.0625 M Tris-HCl, pH 6.8, 8 M urea, 0.5 mM EDTA, 0.001% (w/v) Bromophenol blue, 5% (v/v) 2-mercaptoethanol) and stored at -40 °C. a-h, n Cross-refer to Fig. 1.

* Values for antibody titres and the volume used are given in parentheses.

$\dagger$ 2 ml packed cells used.

Figure 1 shows the results of SDS-PAGE analysis. In preliminary experiments (not shown) we could not obtain discrete electrophoretic separation of ^{125}I -labelled bands using the standard Laemmli sample buffer because the labelled material did not penetrate the stacking gel. Inclusion of 8 M urea in the sample buffer was essential to obtain discrete separation, thus suggesting the hydrophobic nature of these components.

Figure 1 shows that non-specific binding in negative controls (Fig. 1b, d, f, h) was not a problem in our procedure. The ^{125}I activity detected in negative controls (Table 1) was not detected by autoradiography and presumably represents low molecular weight, non-specifically adsorbed material not retained in the gel during fixation as autoradiography readily detects this level of activity^{15,16}. The complexes isolated from cells sensitized with anti-Rh(D), anti-Rh($\bar{c}$) and anti-Rh(E) antibodies gave similar patterns of Coomassie brilliant blue (CBB) staining. In addition to IgG heavy (H) and light (L) chains, all contained CBB-stained components migrating in the band 3 region. This is of interest because recent reports suggest that band 3 is associated with the expression of Rh(D) activity^{12,17}. However we did not detect any radioactivity in these components, suggesting that either these are not of band 3 origin (it is known that band 3 is labelled by the procedure used in this work¹⁸) or they are composed of an unlabelled band 3 subpopulation. We cannot completely exclude the possibility that these bands are derived from IgG.

Marked differences were observed in the radiolabelled components present in the various immune complexes which we isolated; for example compare the anti-Rh(D) complex pattern with that of anti-Fy^a complex (Fig. 1j and i respectively), and the patterns of the anti-Rh($\bar{c}$) and anti-Rh(E) complexes (Fig. 1c and e respectively). Differences were also observed in the Rhesus system; thus anti-Rh(D) complexes contained in addition to the 28,500-MW component (A28 in Fig. 1) common to all Rhesus complexes, bands of 50,000 and 68,000 MW which together accounted for ~20% of the radioactivity in the anti-Rh(D) complex. The presence of these extra bands was not influenced by sample loading (compare Fig. 1a with j). The possibility of qualitative differences between Rh(D)-associated components on -D/-D- and $\bar{c}DE/\bar{c}DE$ cells was investigated. Anti-Rh(D) complexes from these cells were qualitatively identical (Fig. 1n, o). However, we observed an increase in a

component with similar mobility to A28 when comparing Triton extracts of -D/-D- (Fig. 1k) and $\bar{c}DE/\bar{c}DE$ (Fig. 1l) cells. This component is probably A28 because there is increased expression of Rh(D) on -D/-D- cells. The A28 bands were the most prominent feature in the three Rhesus complexes tested and therefore most likely to carry the majority of the antigen sites on the cell surface. The ratio of ^{125}I activity to observed CBB staining intensity for A28 was much higher in anti-Rh(D) complexes. There are two possible explanations: (1) Rh(D) may be associated with a different population of A28 molecules which contain a higher number of iodination sites, and (2) the increased ratio of label to CBB stain may reflect a greater exposure on the red cell surface. The latter possibility is intriguing because it is well documented that Rh(D) is by far the most immunogenic of the Rhesus antigens^{19,20}.

The lower quantity of IgG H and L chains observed in anti-Fy^a complexes (Fig. 1g) may reflect the smaller number of Fy^a sites per cell compared with Rhesus antigens⁵. We concluded from Fig. 1g, m that expression of Fy^a activity is associated with a radiolabelled band of 39,500 MW showing similar mobility to PAS 2 (compare Fig. 1l with m). Labelled bands of 64,000 and 88,000 MW were also seen with counterparts in Triton extracts of labelled membranes (Fig. 1k, l, p). The 88,000-MW component had a similar mobility to PAS 1 and band 3. The possibility that Fy^a is carried by a fraction of the major red cell membrane sialoglycoprotein and band 3 requires further investigation. The 50,000-MW component (Fig. 1i) with an apparent similarity to the 50,000-MW component associated with Rh(D) (Fig. 1j) was not always observed in anti-Fy^a complexes (see Fig. 1m). The identity of this component is therefore obscure.

The molecular composition of the Rhesus and Duffy native antigens remains unclear. Folkler *et al.*²¹ have measured a value of $174,000 \pm 10,000$ for the *in situ* molecular weight of Rh(D) by radiation inactivation, which suggests that A28 is a subunit of a larger molecule or that degradation has occurred during immune complex isolation. Degradation due to radiolabelling or proteolysis was not detected in our procedure. Identical CBB staining of Rh complexes isolated from labelled and unlabelled cells was seen in SDS-PAGE. Identical CBB staining and autoradiographic profile were also seen when 1 mM phenyl-

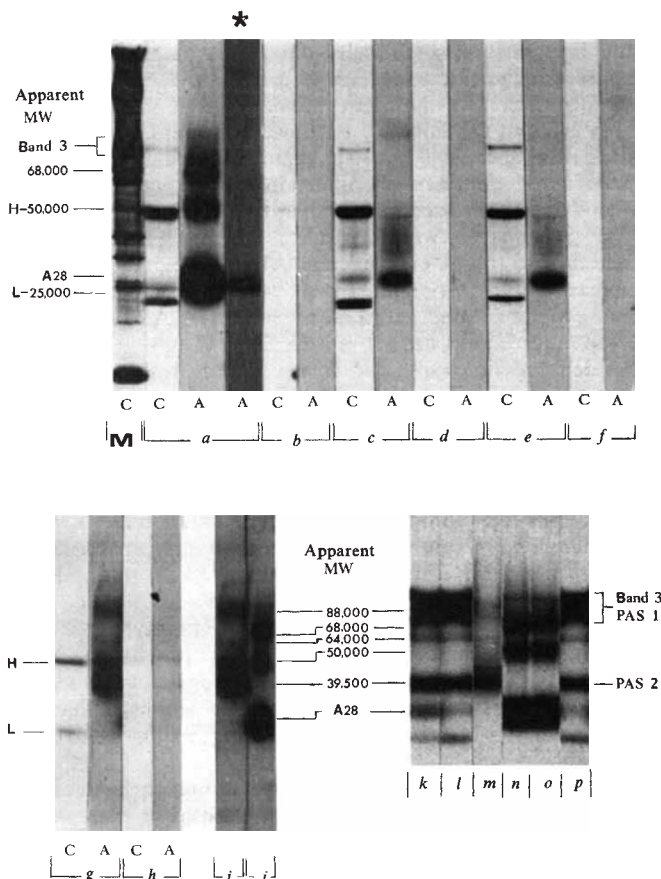


Fig. 1 SDS-PAGE of immune complexes eluted from protein A-Sepharose (see Table 1). Electrophoresis was performed at 35 mA (stacking) and 100 mA (running) on 7.5–15% polyacrylamide gradient gels ($180 \times 140 \times 0.27$ cm; Pharmacia GE2/4LS vertical apparatus) with a 5% polyacrylamide stacking gel using the discontinuous system of Laemmli²⁴. Gels were stained with CBB and autoradiography of dried gels performed at -75°C using Kodak XS-1 X-ray film and Cronex Lighting Plus intensifying screens^{15,16}. Films were developed and fixed with Kodak DX-80 and FX-40 respectively, according to the manufacturers instructions. Samples were incubated overnight at 37°C before electrophoresis. Samples *a–h* (see Table 1) were applied to gels in their entirety. *i, j*, Comparison of autoradiographs of anti-Fy^a (*i*) and anti-Rh(D) (*j*) immune complexes, 20,000 c.p.m. per sample. *k–p*, Autoradiographic comparison of anti-Rh(D) complexes from *-D/-D-* (*n*) and *$\bar{e}$ DE/ $\bar{e}$ DE* (*o*) cells with anti-Fy^a complexes (*m*; separate experiments to *g, i*), and with Triton-soluble membrane fractions sampled before protein A-Sepharose addition and diluted with 9 vol of electrophoresis sample buffer plus 5% SDS; *k, l, n–p*, *$\bar{e}$ DE/ $\bar{e}$ DE*, Fy(a+) cells, 50,000 c.p.m. were applied for samples *k, l, n–p*; 16,000 c.p.m. for *m*. Average c.p.m. for total Triton soluble fraction (7 ml) $\approx 4 \times 10^7$. Autoradiograph exposure times: *a–j, m*, 60 h; *k, l, n–p*, 36 h. H, L, H and L chains of IgG; A, autoradiograph; C, CBB stained. *, Short exposure of Kodak XR-1 film to demonstrate the centre of the radiolabelled band. M, red cell ghosts solubilized in sample buffer plus 5% SDS.

methylsulphonyl fluoride, 2 mM *N*-ethylmaleimide and 0.5 mM EDTA were included in cell lysis and membrane solubilization buffers to inhibit proteolysis. The possibility that A28 and the 68,000-MW component in Rh(D) complexes (Fig. 1*a, j, n, o*) are the band 3 degradation products reported to bind IgG non-specifically¹² can be discounted as these were absent in Fy^a complexes (Fig. 1*i, m*).

In conclusion, the advantages of our procedure are its simplicity, lack of non-specific interference and the ease with which membrane components exposed on the outer surface of the red cell can be identified. Coupled with the potential for preparative isolation of immune complexes we consider this technique to be of value for future work on the structure of red cell antigens.

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Absence of *Ir* gene control of T cells recognizing foreign antigen in the context of allogeneic MHC molecules

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The major histocompatibility complex (MHC)-linked immune response (*Ir*) genes confer high responsiveness on some MHC haplotypes and low or no responsiveness to certain antigens on other haplotypes (for review see ref. 1). The cause of nonresponsiveness is thought to be a failure of T cells to be stimulated by a given combination of antigen and MHC molecules, which can, in principle, arise in two ways: either the antigen-presenting cells (APCs or macrophages) of the nonresponder haplotypes are incapable of presenting the foreign antigen to T cells in an immunogenic form^{2,3} or the T-cell repertoire of nonresponder haplotypes lacks clones recognizing the antigen in the context of a given MHC⁴. We have recently shown that APCs from nonresponder strains can in fact present antigen to allogeneic responder T cells, depleted of alloreactivity against the APCs themselves⁵, which indicates that the mechanism of nonresponsiveness is not a failure of APC function. Using the same experimental system, we show here that responses of T cells to antigen presented on allogeneic APCs do not depend on the *Ir* phenotype of either the T cell or the APC: *Ir* gene control is completely absent from these responses. The results strongly suggest that *Ir* gene-controlled nonresponsiveness is associated with the adjustment of the T-cell repertoire to self-MHC antigens.

Recently we have developed an assay system for measuring the proliferative response of T cells to antigens presented by allogeneic APCs⁵. T cells were prepared by nylon wool-column separation of nucleated spleen cells from unprimed mice, and adherent cells obtained from peritoneal washing served as APCs. The T cells were first cultured with allogeneic APCs and alloreactive T cells were removed by treatment with 5-bromo-2'-deoxyuridine (BUdR) and light on day 3 (ref. 6). The surviving T cells were then primed *in vitro* using fresh allogeneic APCs and an antigen, the response to which is under *Ir* gene control. After an additional 7 days in culture, T cells were collected, distributed into flat-bottomed microtitre plates and tested for

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Table 1 *Ir* gene-controlled secondary proliferation of T cells primed *in vitro* with GA on syngeneic APCs

T cell		Responder status	APC	Δ c.p.m.	SI
Strain	H-2 haplotype				
C57BL/10	<i>b</i>	R	C57BL/10	7,467	45.7
BALB/c	<i>d</i>	R	BALB/c	8,308	3.6
B10.D2	<i>d</i>	R	B10.D2	7,733	23.6
B10.P	<i>p</i>	NR	B10.P	112	1.2
C3H.NB	<i>p</i>	NR	C3H.NB	243	1.4
B10.Q	<i>q</i>	NR	B10.Q	89	1.2
DBA/1	<i>q</i>	NR	DBA/1	43	1.1
A.SW	<i>s</i>	NR	A.SW	-113	0.7
(B10.A(5R)F ₁ × B10.P)	<i>i5</i> × <i>p</i>	R × NR	F ₁	25,363	28.9
(B10.A(5R)F ₁ × B10.P)	<i>i5</i> × <i>p</i>	R × NR	B10.A(5R)	36,679	32.6
(B10.A(5R)F ₁ × B10.P)	<i>i5</i> × <i>p</i>	R × NR	B10.P	416	1.5

Proliferation of *in vitro* primed T cells (1×10^5) in the presence of 1×10^5 APCs and $40 \mu\text{g ml}^{-1}$ GA was measured by ^3H -thymidine incorporation⁷. The results are expressed as Δ c.p.m., that is, c.p.m. in cultures with GA and APC - c.p.m. in cultures with APC and without GA; and stimulation index (SI), that is, c.p.m. in cultures with GA and APC divided by c.p.m. in cultures with APC but without GA. The background c.p.m. in cultures without antigen were in the range 167-3,224. The culture and assay conditions were identical (including preculturing of T cells with APCs alone, followed by BUdR and light treatment) to those used for allogeneic T cell-APC combinations⁵. R, responder; NR, nonresponder.

secondary proliferative response to the priming antigen and fresh APCs in a 3-day assay⁷. In this system, allogeneic APCs alone did not induce appreciable specific T-cell proliferation. Using the antigens poly(Glu⁴⁰Ala⁶⁰) (GA), poly(Glu⁵¹Lys³⁴Tyr¹⁵) (GLT) and lactate dehydrogenase B (LDH-B), secondary T-cell proliferation was induced only by the priming antigen presented on the priming allogeneic APC. Furthermore, proliferation was inhibited by monoclonal antibodies raised against Ia antigens on the APCs, but was not affected by antibodies to Ia antigens present on the T cells (ref. 5 and N.I., unpublished results). Thus, the secondary proliferation *in vitro* of T cells is antigen specific, and is restricted by Ia antigens of the allogeneic APCs.

This study was undertaken to determine the possible influence of the *Ir* phenotype of T cells and APCs on the secondary response *in vitro*. We used GA as a test antigen because the number of responder and nonresponder H-2 haplotypes to this antigen is about the same⁷; this allowed us to assemble a large number of allogeneic T cell-APC combinations with strains of different responder status. To ascertain that our *in vitro* system is subject to the same *Ir* gene control as the *in vitro* response of T cells primed *in vivo*, we first studied the responses of T cells primed *in vitro* to GA on syngeneic APCs. The data in Table 1 demonstrate that responder T cells can mount a proliferative response when primed with GA on syngeneic APCs, whereas nonresponder T cells cannot. T cells from F₁ hybrids between responder and nonresponder mice can only respond to GA when presented by F₁ APCs, or APCs of the responder parent. Thus we conclude that the *Ir* gene control of the *in vitro* secondary T-cell response is identical to that observed after priming *in vivo*^{7,8}. We then tested the anti-GA response in a large number of allogeneic T cell-APC combinations. The combinations fell into four groups: (1) responder T cell-responder APC, (2) nonresponder T cell-nonresponder APC, (3) nonresponder T cell-responder APC, and (4) responder T cell-nonresponder APC. The results (Table 2) show that T-cell proliferation to GA was readily induced in all four types of strain combination. The rate of success of our experiments was 83% (for a total of 108 tests), and the occasional negative results were never reproducible. The data therefore establish that the response of T cells to antigen presented by fully allogeneic APCs is not under *Ir* gene control.

However, note that we did observe nonresponsiveness in partially allogeneic T cell-APC combinations. For example, of several strain combinations where the genetic difference between the T cells and APCs consisted of non-expression as opposed to expression of the I-E molecule (such as B10.A(4R)-B10.A(2R) and B10.S(7R)-(B10.S(9R)), some combinations were consistently found to respond, whereas others were nonresponders, to the antigen GLT. The detailed results of these experiments and their interpretation will be published elsewhere (N.I., Z.A.N. and J.K., in preparation).

Table 2 Lack of *Ir* gene control of the proliferative response of T cells to GA presented by allogeneic APCs

T cell			APC				
Strain	H-2 haplotype	Responder status	Strain	H-2 haplotype	Responder status	Δ c.p.m.	SI
C57BL/10	<i>b</i>	R	BALB/c	<i>d</i>	R	17,534	23.0
C57BL/10	<i>b</i>	R	CBA	<i>k</i>	R	22,773	74.7
B10.D2	<i>d</i>	R	BALB.B	<i>b</i>	R	45,163	34.3
BALB/c	<i>d</i>	R	B10.BR	<i>k</i>	R	14,829	13.3
B10.BR	<i>k</i>	R	BALB/c	<i>d</i>	R	8,701	17.9
B10.BR	<i>k</i>	R	A.CA	<i>f</i>	R	40,848	16.6
C3H.NB	<i>p</i>	NR	B10.WB	<i>j</i>	NR	22,678	9.0
C3H.NB	<i>p</i>	NR	DBA/1	<i>q</i>	NR	17,600	7.0
C3H.NB	<i>p</i>	NR	B10.RIII	<i>r</i>	NR	17,251	7.3
C3H.NB	<i>p</i>	NR	B10.PL	<i>u</i>	NR	24,215	6.9
B10.P	<i>p</i>	NR	B10.PL	<i>u</i>	NR	20,735	10.5
DBA/1	<i>q</i>	NR	C3H.NB	<i>p</i>	NR	11,490	5.5
DBA/1	<i>q</i>	NR	A.SW	<i>s</i>	NR	32,857	15.3
DBA/1	<i>q</i>	NR	B10.SM	<i>v</i>	NR	15,490	13.3
A.SW	<i>s</i>	NR	B10.Q	<i>q</i>	NR	26,465	18.1
C3H.NB	<i>p</i>	NR	BALB.B	<i>b</i>	R	38,224	13.5
C3H.NB	<i>p</i>	NR	BALB/c	<i>d</i>	R	36,833	35.6
C3H.NB	<i>p</i>	NR	A.CA	<i>f</i>	R	27,180	11.0
DBA/1	<i>q</i>	NR	BALB.B	<i>b</i>	R	9,039	4.7
DBA/1	<i>q</i>	NR	DBA/2	<i>d</i>	R	7,398	4.1
DBA/1	<i>q</i>	NR	B10.M	<i>f</i>	R	18,971	26.8
A.SW	<i>s</i>	NR	BALB/c	<i>d</i>	R	11,121	29.2
C57BL/10	<i>b</i>	R	B10.Q	<i>q</i>	NR	11,338	8.4
C57BL/10	<i>b</i>	R	B10.S	<i>s</i>	NR	15,566	36.0
BALB/c	<i>d</i>	R	B10.P	<i>p</i>	NR	17,479	7.8
BALB/c	<i>d</i>	R	B10.Q	<i>q</i>	NR	22,407	14.2
BALB/c	<i>d</i>	R	B10.RIII	<i>r</i>	NR	13,514	6.3
BALB/c	<i>d</i>	R	B10.SM	<i>v</i>	NR	13,039	9.8
A.CA	<i>f</i>	R	B10.Q	<i>q</i>	NR	12,332	9.9

The assay conditions and calculations of Δ c.p.m. and SI were as described in Table 1 legend. Background c.p.m. (cultures with allogeneic APCs but without GA) were in the range 445-4,263.

The lack of *Ir* gene control in allogeneic T cell-APC interactions is best explained by assuming that the T-cell repertoire of each individual consists of (at least) two different compartments: one that recognizes foreign antigen in the context of self-MHC antigens⁹⁻¹³, and another that uses all allogeneic MHC antigens of the species for antigen recognition. The self-restricted part of the repertoire is incomplete, that is, it exhibits *Ir* gene-controlled nonresponsiveness, whereas the allogeneic MHC-restricted part appears to be complete. Nonresponsiveness probably arises by functional or physical elimination of autoaggressive T-cell clones from the self-restricted repertoire¹⁴. This elimination process should not affect the allogeneic MHC-restricted part of the repertoire. The physiological significance of T cells restricted by allogeneic MHC is unclear. There are two possible explanations: either these cells are irrelevant for the individual or allogeneic restriction represents cross-reactivity of T cells normally recognizing another foreign antigen together with self-MHC molecules.

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tRNA precursor transcribed from a mutant human gene inserted into a SV40 vector is processed incorrectly

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We have identified 12 initiator methionine tRNA ($tRNA_i^{Met}$) genes in the haploid human genome¹. One gene, cloned from a recombinant phage library of human fetal liver DNA, contained a base substitution at position 56 in the mature eukaryotic initiator tRNA sequence. The substitution, a G replaced by a T, should generate a tRNA species having a U at a sequence position not observed in any of 178 sequenced prokaryotic and eukaryotic tRNAs². Recently, we have demonstrated³ that this variant tRNA gene is efficiently transcribed in an homologous *in vitro* system derived from KB cells. However, the precursor RNA species of the variant gene, unlike that of the normal one, is not fully processed to a mature sequence in these cell-free extracts. Although the three nucleotides of the 5' leader sequence are efficiently trimmed, the 13-nucleotide 3' trailer is not processed, resulting in the accumulation of an 87-nucleotide precursor. In this report we assay the functional properties of the human variant initiator tRNA gene within an intact cell by studying its transcription from a simian virus 40 (SV40) recombinant during lytic infection of African green monkey kidney (AGMK) cells. We demonstrate that the variant gene is efficiently transcribed when inserted in the SV40 chromosome but, as *in vitro*, the precursor tRNA is not processed to a mature tRNA species.

The SV40 vector used for this study has been described in detail elsewhere⁴. The structure of the SV40- $tRNA_i^{Met}$ recombinant (SVtm) is shown in Fig. 1. It contains a 1.15-kilobase (kb) fragment of human genomic DNA harbouring a single $tRNA_i^{Met}$ gene. This fragment is inserted in an SV40 vector that contains the origin of replication, the entire viral early region, and the extreme 5' and 3' termini of the viral late region. The $tRNA_i^{Met}$ gene is oriented such that its polarity of transcription is opposed to that of the viral late promoters. AGMK cells were infected with a mixture of SVtm DNA and the temperature-sensitive helper, SV40tsA239 (ref. 5), and the resulting viral stocks were used in the following studies.

Confluent flasks of AGMK cells were infected with an innoculum of the viral stock prepared as described above, or with a control preparation consisting of a SV40 recombinant containing murine α -fetoprotein DNA sequences⁶. After 60 h of infection at 39.5 °C, the cells were placed in low phosphate medium containing 100 μ Ci ml⁻¹ of ³²P_i, and cultured for an additional 6 h. Restriction analysis of the DNA recovered from one such infection showed that SVtm represented ~60% of the viral molecules and SV40tsA239 the remainder.

Total cytoplasmic and nuclear RNA were recovered from the infected cells and analysed by electrophoresis in 10% polyacrylamide gels in 7 M urea⁷. An autoradiograph of one such product analysis is shown in Fig. 2. Both the cytoplasmic and nuclear fractions from SVtm-infected cells contain a single low-molecular weight species, absent from the control samples, that is ~89 nucleotides long, compared with the 90-nucleotide primary transcript observed *in vitro*³. This RNA species represents ~30% of the newly synthesized tRNA species resolved on the gel. The relative intensities of the other low-molecular weight RNA species observed do not appreciably differ between the SVtm and control infections. For example, with respect to the intensity of the 5S RNA species, the amounts of labelled 4.5S and total tRNA do not differ between the SVtm- and control-infected cells.

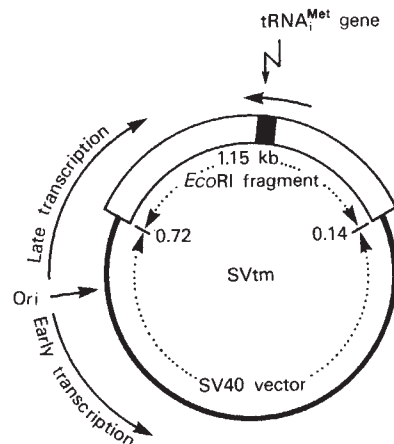


Fig. 1 SV40 recombinant containing the 1.15-kb fragment of human DNA bearing the variant $tRNA_i^{Met}$ gene. A 3.1-kb fragment of SV40 DNA, extending from 0.72 to 0.14 on the circular map and containing the early region, was cloned in pBR322 at the *EcoRI* site as described previously⁴. The pBR322-SV40 vector was linearized by partial *EcoRI* digestion and recovered after agarose gel electrophoresis. The linear vector was ligated to the 1.15-kb fragment bearing the variant $tRNA_i^{Met}$ gene¹, and the ligation mixture used to transform *Escherichia coli* strain LE392 (ref. 18). Clones bearing the 1.15-kb insert were identified by colony hybridization¹⁹, and one containing the $tRNA_i^{Met}$ in an orientation opposite to that of the late viral promoter was propagated. Plasmid DNA from this clone was isolated²⁰ and digested partially with *EcoRI*, releasing a 4.25-kb fragment consisting of the 3.1-kb viral sequence and the 1.15-kb fragment. Following purification by gel electrophoresis, the fragment was recovered and circularized with T4 DNA ligase⁴. Ori, origin of viral replication; ■, schematic location of the $tRNA_i^{Met}$ gene within the chromosomal insert, with arrow above indicating direction of transcription from the tRNA promoter.

The RNA species accumulating in the SVtm infection was recovered from the gel and analysed by classical fingerprinting techniques^{8,9}. A fingerprint of a T1 digest of this RNA is shown in Fig. 3 and the complete sequence is presented in Fig. 4. The numbered spots were identified by secondary complete pancreatic RNase digestion and product analysis. Absolute identification was aided by knowledge of the sequence and previous fingerprint analysis of *in vitro* transcripts of this gene³. The point critical to this discussion is that the transcript is clearly a precursor of the variant tRNA_i^{Met}. The base substitution at position 56 eliminates an AUCG oligonucleotide and results in the appearance of the long oligonucleotide AUCUAAACCAUCCUCUG (spots 12, 13), representing the loop 4 region of the tRNA. The 3'-terminal sequence CUUU is present, along with the oligonucleotide CUAAAG, which lies within the 3' trailer. Digestion of the precursor with T1 generates a pUUAG arising from position -2 in the DNA sequence. Analysis of a total T2 digest by two-dimensional TLC fails to reveal any pppGp.

We have performed only a preliminary analysis of base modification within the precursor. 1-Methylation of adenosine at position 57, adjacent to the base substitution at 56, occurs in ~70% of the precursor. The adenosine at position 36, adjacent to the anticodon, and normally modified in tRNA_i^{Met} to N⁶-(threonylcarbamoyl)adenosine is unmodified in the precursor (data not shown). Analysis of total modified nucleosides by the TLC methods of Nishimura¹⁰ demonstrated some 5,6-dihydrouridine, 5-methylcytidine (position 47) and G-methylation,

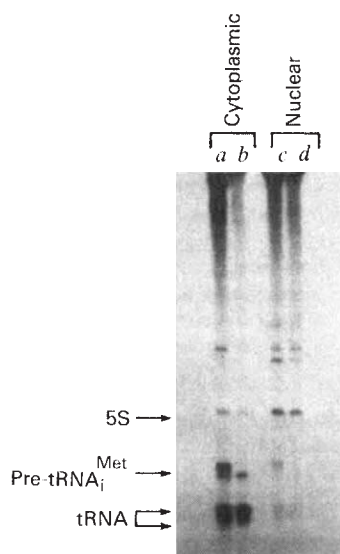


Fig. 2 Electrophoretic analysis of ³²P-labelled RNA isolated from AGMK cells during late phase of SVtm infection. SVtm, described in Fig. 1, was encapsidated by transfection of AGMK cells with 0.5 µg of SVtm DNA and 0.2 µg SV40 tsA239 helper DNA, as described elsewhere⁴; 2.5 ml of the resulting lysate was added to a monolayer of 2 × 10⁷ AGMK cells and incubated at 39.5 °C for 64 h. Media was replaced with minimal essential medium without phosphate but containing 5% dialysed fetal calf serum. Carrier-free ³²P (New England Nuclear) was added to 100 µCi ml⁻¹, and the cells were incubated at 39.5 °C for an additional 6 h. The flasks were washed several times with Dulbecco's phosphate-buffered saline (PBS) (without added magnesium and calcium) and then scraped free with a rubber policeman. After several washes with PBS the cells were washed once in TKM buffer containing 50 mM Tris-HCl pH 7.6, 80 mM KCl, 5 mM magnesium chloride, collected by centrifugation, and lysed by suspension in 1 ml of TKM containing 0.2% NP-40. Nuclei were removed by centrifugation at 10,000g for 10 min. The post-nuclear supernatant was collected, made 0.2% in SDS and extracted twice with 1 vol of phenol, 1/2 vol chloroform/isoamyl alcohol (24:1). Nuclear RNA was extracted by the hot acid-phenol method²¹. A control infection, using the identical SV40 vector ligated to a 2.5-kb fragment containing the murine α-fetoprotein gene sequences⁶ was carried through all corresponding steps. Samples of RNA were loaded onto a 1 mm-thick, 25 cm-long 10% acrylamide gel in TBE buffer⁷ containing 7M urea and electrophoresed at 700 V for 4 h. The gels were autoradiographed against Kodak XR2 film at -20 °C for 20 min. Lanes a, c, ³²P-RNA from cells infected with SVtm; lanes b, d, RNA from cells infected with SV40 vector containing murine α-fetoprotein genomic insert. Arrows indicate mobilities of 5S RNA, tRNA and tRNA_i^{Met} precursor.

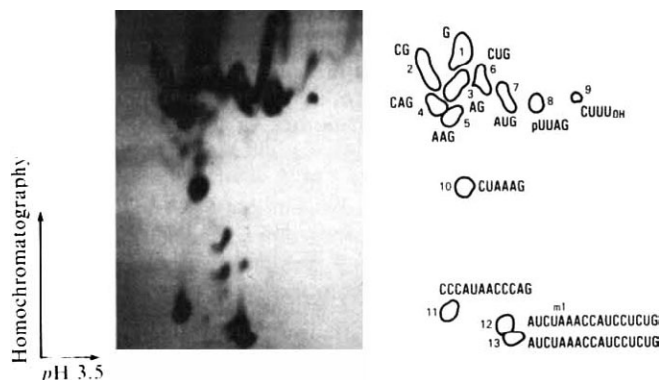


Fig. 3 Fingerprint analysis of the 89-nucleotide RNA accumulating during late infection with SVtm. The major species migrating in the pre-tRNA region, as shown in Fig. 2a, was recovered from the gel²² (10⁵ d.p.m.), digested to completion with T1 RNase and fingerprinted as described elsewhere^{8,9} using iontophoresis on cellulose acetate at pH 3.5 in the first dimension; and homochromatography on DEAE-cellulose TLC plates in the second dimension. Secondary digestions of selected oligonucleotides were done using pancreatic RNase, followed by electrophoresis on DEAE-cellulose paper, to confirm oligonucleotide assignment. 1-Methyladenosine was identified by secondary digestion of oligonucleotide 12 with RNase T2, followed by two-dimensional TLC¹⁰.

but the full extent of these, and specific positions, have not been determined.

As the precursor represents ~30% of the total newly synthesized RNA in the tRNA population, we would expect that efficient processing of this species should result in the accumulation of tRNA_i^{Met} as a major RNA species in the cell. However, hybridization of equal amounts of gel-purified tRNA from SVtm and control infections to filter-bound tRNA_i^{Met} DNA occurred at approximately equal rates and reached a maximum level of hybridization of ~1% of the input radioactivity in each case (data not shown). We conclude that conversion of the mutant precursor to mature tRNA does not occur with any significant efficiency.

Conclusions concerning the *in vivo* activity of the human variant tRNA_i^{Met} gene are based on the assumption that transcription of the gene occurs from the tRNA_i^{Met} promoter. Several observations lead us to believe that this is the case. The tRNA_i^{Met} is most likely transcribed from the recombinant SV40 chromosome by RNA polymerase III, based on the 3'-terminal sequence of the tRNA_i^{Met} precursor, GCUUU. The polymerase responsible appears to have recognized accurately the termination signal of the gene, GCTTTT..., a property, as far as we know, unique to RNA polymerase III¹¹. Although the major intermediate lacks a 5'-terminal triphosphate, and thus is not an intact initial transcript, it is shorter by only one nucleotide than the primary transcript observed during transcription *in vitro* of the variant tRNA_i^{Met} gene. We presume that some nibbling of the 5' end has occurred in the system, similar to that observed during the biosynthesis of tRNA^{Tyr} following injection of the gene into the intact *Xenopus laevis* oocyte¹². Although it is possible that the 89-nucleotide intermediate represents the product of previous cleavage of a longer RNA species initiated upstream, we believe there is no strong evidence to support this possibility. No RNA polymerase III promoter has been demonstrated on the 1.15-kb chromosomal fragment inserted in SV40, except for the tRNA_i^{Met} gene, in *in vitro* systems using KB cells and *X. laevis* ovarian extracts³. Initiation from within a viral sequence is unlikely as no functional RNA polymerase III transcription unit having activity comparable with that observed on the SVtm recombinant has been identified on SV40¹³. Furthermore, the level of expression is much higher than would be expected from the viral early promoter, which is oriented in the same polarity as the tRNA gene, representing no more than 0.01% of total RNA synthesized late in lytic infections¹⁴.

Although the variant human tRNA_i^{Met} gene is transcribed *in*

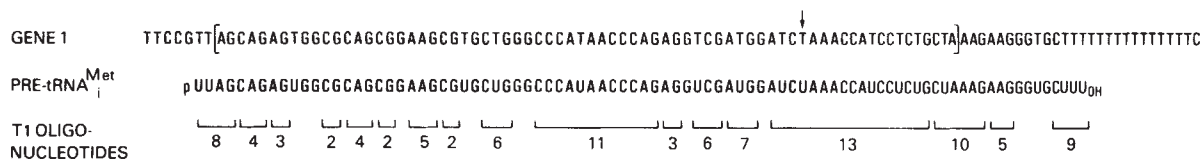


Fig. 4 Sequence of the variant tRNA^{Met}_i transcript and the corresponding gene. Arrow denotes the base transversion at position 56 in the tRNA^{Met}_i sequence. Brackets indicate mature termini of the tRNA. Numbered oligonucleotides correspond to those noted in Fig. 3. The sequence of the gene and flanking regions was determined previously¹.

Our finding that the variant human tRNA^{Met} gene is transcribed in an intact cell but that the precursor is not efficiently processed to a mature tRNA suggests that mutations within eukaryotic tRNA genes may be more prevalent than assumed from the limited sequence variation observed in mature tRNA species. We suggest that nucleolytic processing of tRNA precursors, which in the case of the human tRNAⁱ_{Met} gene transcript involves excision of a 5' leader and 3' trailer, provides a 'proof-reading' function resulting from stringent sequence or structural

Although it is clear that the variant tRNA_i^{Met} gene is expressed on the SV40 vector, it does not necessarily follow that the gene is active in the human genome. Foreign genes inserted into SV40 are generally expressed regardless of the state of transcriptional activity of the gene in the infected cell¹⁶. We do not yet know the functional state of the variant tRNA_i^{Met} locus in human somatic cells, although several studies probing the nuclease sensitivity¹⁷ of the locus are in progress.

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Stereochemistry of iron in deoxyhaemoglobin

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X-ray crystallography has shown that in deoxyhaemoglobin the iron atoms are displaced by 0.56 ± 0.03 Å from the mean porphyrin plane, whereas in liganded haemoglobins they lie either in or close to that plane^{1,2}. Perutz³ proposed this shift as one principal factor responsible for haem-haem interaction. Eisenberger *et al.* used extended X-ray absorption fine structure (EXAFS) to measure the Fe-N distance in deoxyhaemoglobin and concluded that the irons lie only $0.2^{+0.1}_{-0.2}$ Å from the plane of the porphyrin nitrogens and that the mechanism of Perutz is therefore invalid⁴. We have now compared the EXAFS of deoxyhaemoglobin with that of the ferrous 'picket fence' 2-methylimidazole complex in which the displacement of the iron from the plane of the porphyrin nitrogens is known to be 0.399 ± 0.004 and 0.426 ± 0.004 Å from the mean porphyrin plane (ref. 5). The two EXAFS spectra are very similar, consistent with similar displacements of the irons. We find the same Fe-N distance of 2.06 ± 0.01 Å in deoxyhaemoglobin as Eisenberger *et al.*, but show that the displacement of the iron cannot be calculated from that distance.

The samples were prepared as follows. A 15 mM solution of human deoxyhaemoglobin was prepared from a red cell lysate by pressure dialysis followed by deoxygenation and addition of neutral $\text{Na}_2\text{S}_2\text{O}_4$ in an oxygen-free glove box. A drop of this solution was mounted in a hole 5 mm wide in a 1 mm-thick copper strip, sealed between thin mylar films and kept under nitrogen before use. The crystalline picket fence complex (2-methylimidazole-*meso*-tetra($\alpha,\alpha,\alpha,\alpha$ -*O*-pivamidophenyl)porphyrinatoiron-(II)-ethanol $[\text{Fe}^{2+}(\text{T piv PP})-(2\text{-MeIm})\cdot\text{EtOH}]$) was prepared in oxygen-free conditions as described in ref. 5. In the first of the two independent EXAFS experiments, 2 mm-wide thin-walled glass capillaries were filled with the sample and sealed with bees' wax. In the second, the complex was diluted by mixing with boron nitride ($\sim 1:1$ by wt) and then formed into a pellet 0.4 mm thick by pressing between two Mylar sheets separated by an aluminium spacer. After the EXAFS experiment, the sample was extracted from the pellet with toluene and checked spectrophotometrically to ensure that neither oxygenation nor oxidation has occurred.

EXAFS associated with the Fe K-edge was recorded both in fluorescence and transmission modes at the Stanford Radiation Laboratory. Five spectra of deoxyhaemoglobin collected at 10K (three scans), 44K (one scan) and 110K (one scan) were averaged to give a good-quality spectrum. For the first picket fence sample a single spectrum was recorded at 100K; for the second sample three spectra were recorded at 300K. No appreciable difference was found between these spectra at $E < 150$ eV. The spectrum analysed in detail at Daresbury was that recorded at 100K; those recorded at 300K were analysed at Stanford.

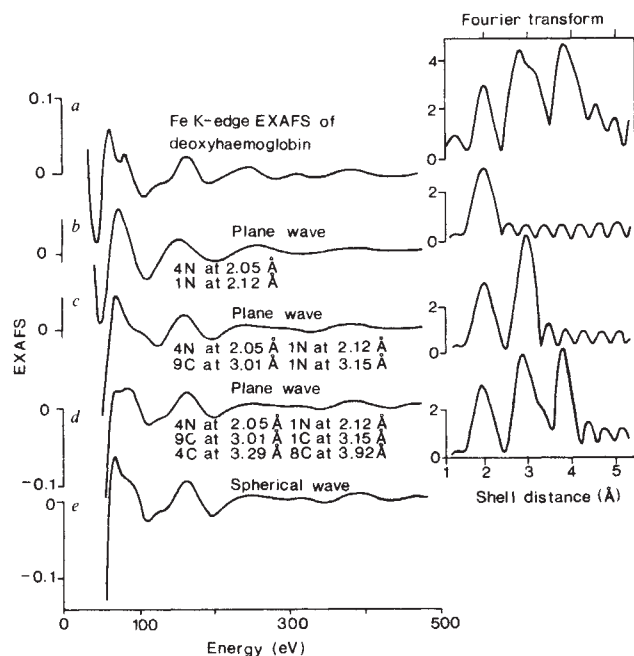


Fig. 1 EXAFS and Fourier transforms of human deoxyhaemoglobin. *a*, Experimental curve and transform derived from it. *b*, Calculated curves taking into account scattering from nearest neighbours only (first shell); *c*, same for first and second shell; *d*, same for first three shells; *b-d* were calculated using the plane wave method. *e*, EXAFS curve calculated for first three shells using spherical wave method. Best-fitting distances of carbon and nitrogen atoms from the iron are listed in Table 1. Fourier transforms have been multiplied by R^2 to obtain the radial intensity distributions of the atoms surrounding the iron.

We have used the atomic coordinates of the picket fence model⁵ to calculate the distances of carbon and nitrogen atoms up to 4 Å from the Fe atom. For data manipulation and the calculation of theoretical spectra at the Daresbury Laboratory we used the procedures and programs described in refs 6 and 7 and for our interpretation we relied on Lee and Pendry's theoretical approach^{8,9}.

Phase shifts were calculated using the MUFPO program, by constructing a complex muffin-tin potential following the standard Matheiss prescription, and making use of the Clementi-Roetti wave functions for carbon and nitrogen atoms in the neutral state. The excited iron atom was approximated by the wave functions of the Co atom with one electron from the 1s orbital removed. The EXAFS spectra of deoxyhaemoglobin and the picket fence are shown in Figs 1-3. They show a close resemblance: each contains several beat regions at 40-90, 100-180 and 200-300 eV, indicating similarities in the distances and nature of the atoms surrounding the Fe atom. The large number of beats suggests that the complex EXAFS profile arises from the interference of several back-reflected waves. This is evident from the Fourier transforms which show strong contributions from scattering distances of up to 4 Å.

The theoretical and experimental EXAFS spectra were converted into k space defined by $k = \sqrt{2(E - E_0)}$ where E is the energy of the incident beam and E_0 the energy of the beam at the absorption edge of iron. Fourier transforms were then calculated with respect to $\exp(-i2kR)$ where R is the radial distance of an atom from the Fe atom. The problems of secondary maxima and changes in peak positions induced by phase shifts were dealt with by correcting the spectrum for the back-scattering factor of the nearest shell and by multiplying the EXAFS spectrum with a gaussian function, $\exp[-0.1(k - k_0)^2]$ with k_0 at the centre of the available range. Instead of using a

Fourier filtering technique and isolating individual shells (peaks) we preferred to identify the contribution of individual shells to the EXAFS spectrum and to the Fourier transforms. This approach has recently been applied successfully to Cu-Zn superoxide dismutase and its various substituents⁷. EXAFS simulations were performed with both the raw and the splined data.

The following parameters were used to calculate the EXAFS: (1) the distances of the shells from the central Fe atom; (2) the kinds and numbers of atoms in each shell; (3) their thermal and structural disorder; and (4) the shift in energy of the X-ray absorption edge (E_0). Parameters (1) and (2) determine the phase and amplitude over the whole energy range; (3) influences the amplitude at energies >200 eV; and (4) linearly shifts the experimental spectrum. Parameters (2) are known. The crystallographic distances in the picket fence⁵ were used as the starting distances for both deoxyhaemoglobin and the picket fence. Parameters (1) and (3) were optimized individually for each shell by visual inspection of (a) the agreement between the experimental and theoretical EXAFS; (b) the agreement between their respective Fourier transforms; (c) by considering the major features of the Fourier transform of the difference between the experimental and theoretical EXAFS; and (d) by monitoring the difference function $[0.15(\chi_{\text{exp}} - \chi_{\text{theo}})]^2$. Once the theoretical EXAFS resembled the experimental one, an edge shift of 15 eV was applied and then parameters (1) and (3) were further refined, again using criteria (a)-(d).

Figure 1 shows the uniqueness of the EXAFS spectrum and its Fourier transform with the contributions to them by the individual shells of atoms. The three beat regions could be simulated only by including all the atoms lying within 4 Å of the Fe atom. Figure 2 shows two theoretical spectra and two theoretical transforms, one set calculated with the optimized Fe-N_{porphyrin} distance of 2.05 Å and the other with a distance of 2.07 Å. The longer distance makes the theoretical curve in the first beat region narrower than the experimental curve and makes the first shell peak of the Fourier transform appear at a longer distance than the experimental one. Figure 3 shows the EXAFS spectrum of the picket fence and its theoretical simulation together with their respective transforms. Table 1 lists the distances and Debye-Waller terms used for the simulations. The most important parameter is the distance from the Fe atom to the porphyrin nitrogens, Fe-N_{porphyrin}. That distance measured by EXAFS at ≤ 110 K is 2.05 (± 0.01) Å for deoxyhaemoglobin and 2.06 ± 0.02 Å for the picket fence; at 300 K a distance of 2.10 ± 0.04 Å was measured for the picket fence: also at 300 K a

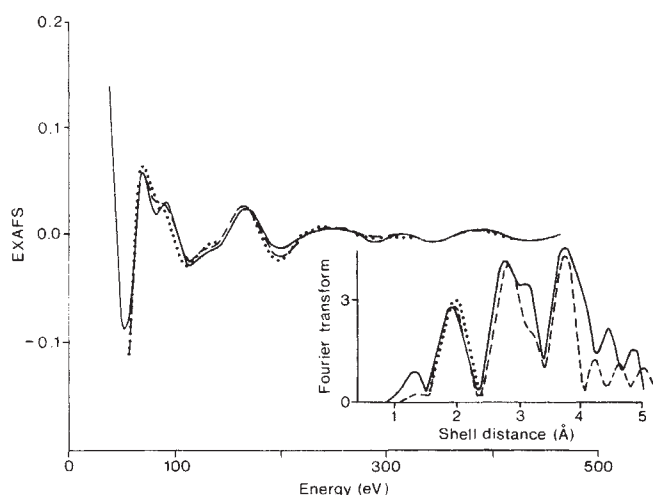


Fig. 2 EXAFS and Fourier transforms of human deoxyhaemoglobin. Theory *a* is the same as shown in Fig. 1*e*; theory *b* was obtained with Fe-N_p distance of 2.07 Å; all other parameters as for Fig. 1*e*. Experimental spectrum is shifted to higher energy: $E_0 = 15$ eV. ---, Theory *a*; ····, theory *b*; —, experiment.

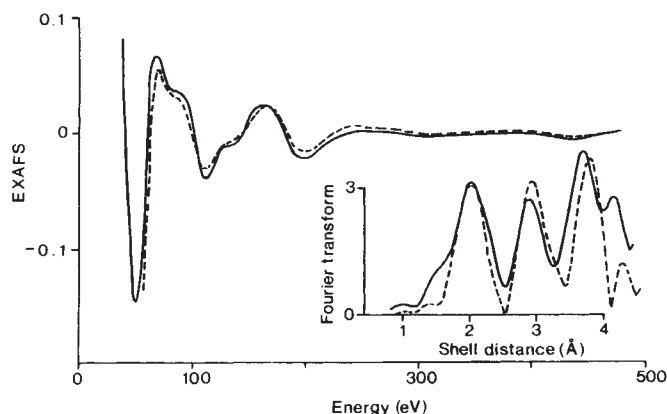


Fig. 3 EXAFS and Fourier transform of oxygen-free picket fence complex (first samples, 100K). Curves were calculated by the spherical wave method. Experimental spectrum is shifted to higher energy: $E_0 = 15$ eV.

distance of 2.072 ± 0.005 Å was found by single crystal X-ray analysis⁵. Thermal expansion between 100K and 300K would lengthen the Fe–N bond by ~ 0.01 Å. All the values therefore agree within the error limits. For the next three shells the agreement between the picket fence EXAFS and crystallographic data is also good, but for the distant shells 4C (3.45 Å) and 8C (4.3 Å), EXAFS gives shorter distances than the crystallographic analysis. A similar apparent shortening of distances was found in the Cu–Zn superoxide dismutase for both the enzyme systems and the well characterized chemical system $\text{Cu}(\text{imid})_4(\text{NO}_3)_2$ (ref. 7). The reasons for this discrepancy were not well understood, but multiple scattering and shadowing effects may become important for distances > 3.2 Å. The application of EXAFS to this well characterized model of deoxyhaemoglobin provides confidence that the theory can be used reliably for determining the distances of atoms within 3.2 Å from the Fe atom; beyond this EXAFS may underestimate them.

Before comparing the EXAFS results with the crystallographic data for deoxyhaemoglobin¹ and the picket-fence

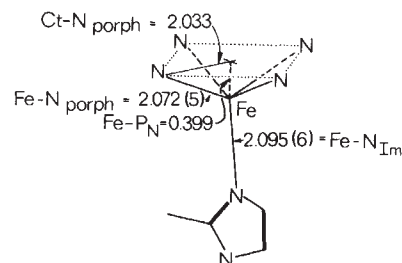


Fig. 4 Stereochemistry of iron coordination in the oxygen-free picket fence complex⁵. Distances in ångströms; numbers in parentheses give the standard error in 0.001 Å.

In the picket fence the four pyrrol rings are domed slightly towards the Fe which lies 0.399 Å from the plane of the porphyrin nitrogens and 0.426 Å from the mean plane of the 24 porphyrin nitrogens and carbons (Fig. 4). In deoxyhaemoglobin the resolution is insufficient to determine whether the pyrrol rings are domed. The distance of the Fe from the planar skeleton of 32 nitrogens and carbons that Fermi fitted to the electron density was 0.56 Å in both the α and β subunits¹. Our EXAFS results are consistent with Fermi's finding as they show that the distances from the Fe atom to the nitrogens and carbons are similar in the picket fence and in deoxyhaemoglobin. Our EXAFS spectrum and Fe–N_{porph} distances agree with those of Eisenberger *et al.*⁴; we wondered, therefore, what led them to conclude that the iron in deoxyhaemoglobin was only 0.2 Å from the porphyrin plane.

Eisenberger *et al.* obtained this value by triangulation. They used their EXAFS distance of 2.055 Å from the Fe to the porphyrin nitrogens (Fe–N_{porph}) in deoxyhaemoglobin as the base of a right-angled triangle; for one of its sides they used the distance of 2.045 Å of the porphyrin nitrogens from the centre of the porphyrin ring (Ct–N_{porph}) found in a single crystal study of model compound $\text{Fe}^{2+}(\text{TPP})(2\text{-MeIm})\cdot\text{EtOH}$ (D. A. Buckingham, quoted in ref. 14). This makes the other side of the triangle $\text{Fe–P}_N = \sqrt{(2.055)^2 - (2.045)^2} = 0.2$ Å. Eisenberger *et al.* also compared the EXAFS of deoxyhaemoglobin with that of a deoxygenated picket fence complex having a water molecule weakly coordinated to the sixth position at the Fe atom. This has $\text{Fe–N}_{\text{porph}} = 2.068 \pm 0.014$ Å, the same as the picket fence we have used, but due to the sixth ligand the displacement of the Fe from the plane of the porphyrin nitrogens is reduced to 0.28 Å (ref. 12).

Table 1 Parameters used to simulate the EXAFS associated with the Fe K-edge of deoxyhaemoglobin and deoxy picket fence complex

Atom	Deoxyhaemoglobin		Deoxy 'picket fence'		Single crystal X-ray analysis ⁵
	σ^2 (Å ²)	R (Å)	σ^2 (Å ²)	R (Å)	
4N	0.004	2.05	0.006	2.06	2.07
1N	0.006	2.12	0.006	2.11	2.10
9C	0.003	3.01	0.011	3.01	3.09
1C	0.003	3.15	0.003	3.15	3.14
4C*	0.003	3.29	0.008	3.34	3.45
8C*	0.003	3.92	0.006	3.89	4.30

R denotes the distance of the atom from the Fe atom. σ^2 is a Debye Waller factor and is equivalent to ΔR^2 . $E_0 = 15$ eV. The difference in Debye Waller factors of the 9 carbon atoms is significant and derives from a difference of EXAFS at ~ 300 eV and of the amplitudes of the Fourier terms at ~ 3 Å (Figs 2, 3).

Table 2 Parameters of five-coordinated high-spin iron porphyrins from single crystal X-ray analyses

Complex	Fe-P _N (Å)	Fe-N _{porph} (Å)	Ct-N _{porph}	Δ	Fe-P _{CN}
O[Fe ³⁺ (diMeOEP)] ₂	0.512	2.065	1.995	0.018	0.535
O[Fe ³⁺ (TPP)] ₂	0.496	2.087	2.027	0.044	0.532
Fe ³⁺ (TPP)(Br)	0.489	2.069	1.992	0.070	0.539
Fe ³⁺ (TPP)(NCS)	0.485	2.065	2.007	0.068	0.541
Fe ³⁺ (TPP)(I)	0.480	2.066	1.996	0.068	0.537
Fe ³⁺ (Protoporph)Cl	0.474	2.062	2.007	0.080	0.541
Fe ³⁺ (Meso-DME)(OCH ₃)	0.456	2.073	2.022	0.034	0.484
Fe ³⁺ (Proto-DME)(SR)	0.434	2.064	2.015	0.015	0.446
Fe ²⁺ (TPP)(2-MeIm)	0.418	2.086	2.043	0.134	0.530
Fe ²⁺ (Tpiv PP)(2-MeIm)	0.399	2.072	2.033	0.027	0.505
Fe ³⁺ (TPP)Cl	0.404	2.063	2.023	-0.015	0.392
Mean	0.457	2.070	2.015	0.052	0.507

P_N, mean plane of porphyrin nitrogens; P_C, mean plane of conjugated carbons; P_{CN}, mean plane of nitrogens and carbons. Δ is the doming parameter, giving the difference between the plane of the nitrogens and carbons (=P_C-P_N). For details of these structures see ref. 15, except Fe²⁺(TpivPP)(2-MeIm) EtOH which is described in ref. 5.

Table 2 summarizes structural parameters for various high-spin iron porphyrins determined by X-ray analysis of single crystals at high resolution. In no case is Fe-P_N < 0.4 Å. If the displacement in deoxyhaemoglobin were only 0.2 Å, as Eisenberger *et al.* proposed, then its stereochemistry would be unique among five-coordinated high-spin iron porphyrins. The triangulation of Eisenberger *et al.* relies on taking the root of the difference of two squares of nearly equal value. A change in Fe-N_{porph} or Ct-N_{porph} of only 0.01 Å therefore changes Fe-P_N by ~0.05 Å. Eisenberger *et al.*'s value of Fe-P_N = 0.2 Å was derived by using Fe-N_{porph} and Ct-N_{porph} values from two different haem complexes. Because calculation shows that $\delta(\text{Fe-P}_N)/\delta(\text{Ct-N}_{\text{porph}}) = 5$, an accurate value of Fe-P_N could be obtained by this method only if Ct-N_{porph} distances were invariant among five-coordinated high-spin haems and a direct correlation therefore existed between Fe-N_{porph} and Fe-P_N; but this is not the case (Table 2 and Fig. 5). The structures of five-coordinated high-spin iron porphyrins determined by X-ray analysis of single crystals at high resolution show that the value of Fe-N_{porph} from one compound cannot be combined with that of Ct-N_{porph} from another to derive a reliable value for Fe-P_N, because the individual distances are too variable and there is no correlation between the two parameters. We conclude that it is impossible to infer from EXAFS the displacement of the iron from the porphyrin plane unless multiple scattering theory can

be applied to the near-edge structure¹³; it has to be measured directly by single crystal X-ray analysis.

When M.F.P. first proposed his stereochemical mechanism of haem-haem interaction, he believed the displacement of the Fe from the porphyrin plane in deoxyhaemoglobin to be due entirely to the increase in iron radius on going from its low-spin in oxyhaemoglobin to its high-spin form in deoxyhaemoglobin. Warshel¹³ and Eisenberger *et al.*⁴ have pointed out that this was an oversimplification. In reality, a mixture of electronic factors (repulsion of the occupied d_{x²-y²} orbitals of the Fe by the π orbital of the porphyrin) and steric factors (van der Waals' repulsion of the proximal histidine by the porphyrin nitrogens) conspire to push the Fe out of the porphyrin plane and dome the pyrroles towards the Fe, so that the Fe atoms and the proximal histidines in deoxyhaemoglobin are further from the mean porphyrin planes than in liganded haemoglobins. However, from the mechanistic point of view, the causes of the displacements do not matter: what matters is that they do occur. Hopfield¹⁴ predicted that the free energy of haem-haem interaction may be directly proportional to the sum of those displacements. Many other observations point to the crucial part played by the movement of the iron and the proximal histidine relative to the porphyrin and the general dominance of stereochemical factors in the mechanism of cooperativity.

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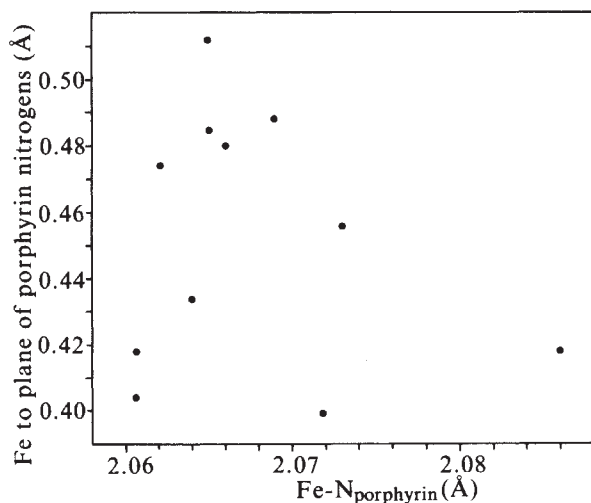


Fig. 5 Mean distances from Fe to porphyrin nitrogen (Fe-N_{porph}) plotted against displacement of Fe from the mean porphyrin plane in five-coordinated high-spin iron porphyrins. Data taken from Table 2.

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ANNOUNCEMENTS

Awards

The National Academy of Sciences awards for 1982 include: James Murray Luck Prize to **Victor A. McKusick**, (The Johns Hopkins School of Medicine); Award in Chemical Sciences to **Gilbert Stork** (Columbia University); Award for Initiatives in Research to **Kerry E. Sieh** (California Institute of Technology); Public, Welfare Medal to **Paul G. Rogers** (formerly of the House Subcommittee on Health and the Environment); Gilbert Morgan Smith Medal to **Luigi Provasoli** (Yale University); Mary Clark Thompson Medal to **William A. Berggren** (Woods Hole Oceanographic Institution); US Steel Foundation Award in Molecular Biology to **Joan A. Steitz** (Yale University); Selman A. Waksman Award to **I.C. Gunsalus** (University of Illinois); Charles Doolittle Walcott Medal to **Martin F. Glaessner** (University of Adelaide); G.K. Warren Prize to **John T. Hack** (The George Washington University); James Craig Watson Medal to **Stanton J. Peale** (University of California).

Dr Theodore Brewster Taylor (Appropriate Solar Technology Institute, Inc.) is the recipient of The Boris Pregel Award for Applied Science and Technology.

Professor William Trager (Rockefeller University) has been awarded the First Rameshwardas Birla Triennial International Award in tropical medicine by the Medical Research Centre of the Bombay Hospital Trust.

Nineteen Prize Fellow selected by D. and Catherine T. MacArthur Foundation will receive unrestricted awards of \$24,000 to \$60,000 annually for five years: **Dr Hugh John Cairns** (Cancer research; microbiology); **Joel E. Cohen** (Mathematics & demography; public health); **Richard P. Critchfield** (Journalism); **Howard E. Gardner** (Developmental psychology; clinician); **John P. Gaventa** (Political science; social activism); **David Hawkins** (Philosophy; education); **John P. Holdren** (Physics; energy and environment); **Ada Louise Huxtable** (Architecture criticism); **Robert W. Kates** (Geography; natural hazards research); **Dr Raphael C. Lee** (Surgery; biomedical engineering); **Cormac McCarthy** (Novels); **Richard C. Mulligan** (Cancer research; biochemistry); **Elaine H. Pagels** (History of religion); **David Pingree** (History of science); **Paul G. Richards** (Theoretical seismology); **Richard M. Rorty** (Philosophy); **Joseph H. Taylor** (Radio astronomy; astrophysics); **Michael Woodford** (Economics; legal theory); **George Zweig** (Physics; neurobiology). In addition Barbara McClintock, 79, a geneticist, has been selected as the Foundation's first Prize Fellow Laureate — assuring her of \$60,000 annually.

Dr Nien-Chu C. Yang (University of Chicago) is the recipient of The Gregory and Freda Halpern Award in Photochemistry sponsored by the Polychrome Corporation.

The Institute of Physics Award for 1982 are: Charles Vernon Boys Prize to **Dr B.J. Isherwood** (GEC Hirst Research Centre); Duddell Medal and Prize to **S. van der Meer** (CERN); Glazebrook Medal and Prize to **Professor J.M.A. Lenihan** (West of Scotland Health Boards); Guthrie Medal and Prize to **Sir Charles Frank** (University of Bristol); Maxwell Medal and Prize to **Dr J.R. Ellis** (CERN); Rutherford Medal and Prize to **Dr D.M. Brink** (University of Oxford); Prizes for the 1981 Graduateship Examination of the Institute to **D.J. Pointer** and **T.J. Mays** (Bristol Polytechnic).

The Paul-Martini-Prize for promoting the development of scientific methods for the evaluation in the clinical pharmacological and therapeutical fields will be conferred by the Paul-Martini-Foundation which is sponsored by the Medizinisch Pharmazeutische Studiengesellschaft (Mainz, FRG). Papers must be self-contained not older than two years. They should be submitted either in English or German with six copies to the Medizinisch Pharmazeutische Studiengesellschaft e.V., Bilhildisstraße 2, Postfach 3048, D-6500 Mainz, FRG before 30 April 1982.

An open competition in geometry for an award of \$2,500 is being sponsored by Science Software Systems, Inc. The award will be presented to the competitor who submits the most complete solution to the problem: What geometrical properties of dissimilar inversion loci are consequences of the property of self-inversion of a basis curve at angles other than 0° or 180°? For further information send a stamped self-addressed envelope to Science Software Systems, Inc., 11899 W. Pico Boulevard, Los Angeles, California 90064, USA.

The Trustees of the Lady Tata Memorial Trust invite applications for Awards for personal support for research on leukaemia, in the Academic Year beginning 1 October 1982. Candidates with programmes of research on any aspect of malignant disease which may throw light on problems of leukaemia will be eligible for consideration. Awards are open to suitably qualified investigators of any nationality, working either in their own institutions or in other centres abroad. Further particulars from the Secretary of the (European) Scientific Advisory Committee, Lady Tata Memorial Trust, MRC Leukaemia Unit, Royal Postgraduate Medical School, Du Cane Road, London W12, UK.

The Eli Lilly Fellowship in diabetes in children has been established by the British Diabetic Association. It will be funded by Eli Lilly & Company. This fellowship will be for a doctor of registrar status and will last for two years. The research into diabetes in children will be carried out at the hospital of the successful candidate. Application forms from the assistant secretary, British Diabetic Association, 10 Queen Anne Street, London W1, UK.

The L.S.B. Leakey Trust is now able to award research grants to scholars in the fields of human palaeontology, archaeology and primate behaviour. Contact: Miss Anne Turner, The L.S.B. Leakey Trust, 10 St George's Mansions, Vauxhall Bridge Road, London SW1, UK.

Appointments

Mr J.W. Bundy (Agricultural General Manager of Birds Eye Foods Limited) has been elected by the Council of the National Institute of Agricultural Botany to be their Chairman for 1982–83.

Professor C.S. Holling (University of British Columbia in Vancouver) will be the new director of the International Institute for Applied Systems Analysis in Laxenburg near Vienna, Austria.

Meetings

1-4 March, **Mobility and Function in Proteins and Nucleic Acids**, London (The Ciba Foundation, 41 Portland Place, London W1, UK).

4 March, **Developments in Low-temperature Biochemistry and Biology**, London (The Royal Society, 6 Carlton House Terrace, London SW1, UK).

15-17 March, **Energy and Feedstocks for the European Chemical Industry**, Belgium (Society of Chemical Industry, 14-15 Belgrave Square, London SW1, UK).

15-23 March, **Introduction of Ultramicrotomy**, Seefelder (Reichert-Jung UK, 820 Yeovil Rd, Slough, UK).

18 March, **The "Simple Surface"**, London (Conference Secretary, IEETE, 2 Savoy Hill, London WC2, UK).

19, March, **Wind and its Effects on Man, Plants and Animals**, Edinburgh (The Royal Society of Edinburgh, 29 Queen St, Edinburgh, UK).

22 March, **European Symposium on the CANDU Reactor**, London (Scientific and Technical Studies, Norwich House, Norwich St, London EC4, UK).

24 March, **Concrete for Durability**, London (The Concrete Society, Terminal House, Grosvenor Gardens, London SW1, UK).

CORRESPONDENCE

Continued from page 452

During a relatively short period, many of the above demands were introduced practically although not by law. In particular the presidium of the academy and the chancellors and deans in almost all universities were chosen in a truly democratic way.

It is significant that most of those elected were politically inactive before 1980 and of moderate views, and gained their authority solely on the basis of their scientific achievements. Thus the elected people can be seen as truly representative of a scientific community which is not heroic but truthful, which follows directives but recognizes that the political direction of science has damaging effects.

It can be argued that a similar attitude is typical of "normal" scientific communities. But the Polish one is not "normal". Its older members endured the Nazi holocaust or harsh life in remote Soviet provinces; their students started their careers during the period of the Stalin cult with its brainwashing machinery. This community suffered the wave of repressions and purges in 1968 followed by the liquidation of the remnants of academic freedoms. It was corrupted during the past ten years of mild but effective "sovietization". Thus it was encouraging to see that the old ideals of scientific freedom, so often declared to be outdated, are so deeply rooted in the bulk of this community and that it is ready to support democratic changes.

Surely the power of the reformers of the scientific establishment rested not only, and not even mainly, on the social forces in their own home but on the forces of an awakening society.

Perhaps much more could have been achieved. There were too many useless meetings, endless and inconclusive discussions about the new structure of science or about formulations of new constitutions. We were drunk after taking too large a draught of democracy and were not yet ready to use it effectively.

Most of the staff of the academy and universities joined Solidarity. But surprisingly, during 1980–81, students were less eager to follow this movement, although a large number of new student organizations appeared. Even the most influential of these, *Niezależne Zrzeszenie Studentów* (NZS, Independent Student Association) was joined by fewer than 20 per cent of the students.

The main action of NZS was the proclamation in October 1981 of a general strike in protest at the halting of legislative procedures concerned with the new progressive law of higher education and in support of the protest of the Radom branch of NZS and Solidarity against the way the rector of Radom Polytechnic had been elected. This strike was joined by a most of the schools of higher education in the country. In spite of the efforts of Solidarity, the Church and the Council of Chancellors of the Polish Schools of Higher Education, this strike was continued after the strikers' first demand was conceded. A few days before martial law was declared the strike broke down. In January 1982 NZS was dissolved by the authorities.

The era of Solidarity — understood here not as a name of this particular organization with its achievements and follies but as synonymous with the greatest all-national movement in our

history — is over. Solidarity is for the time being defeated. But it should be underlined that to the same or an even larger extent the communists who ruled the country for more than 35 years have also been defeated. The political regime proved itself to be not only ineffective and acting against the will of the people, but irreparable and wholly dependent on external powers. In a sense only the Russians won the battle, by finding a way of solving the problem without needing to use their own troops.

The battle is lost, the war is not. It is still the common belief that something very important happened which will influence our future, and perhaps not only ours. I have in mind not only economic and social changes but the changes of spirit of those who rule and those who are ruled — these changes are not as abstract as the word spirit suggests.

Just after the declaration of martial law there was a wave of protests. This wave did not bypass scientific institutions. As yet we do not have a full account of what happened, but we know about the strikes of a few institutes of the academy in the Staszic Palace in the centre of Warsaw which were terminated by force; about a letter of protest signed by more than 300 scientists in the Institute of Chemical Physics; about the strike in the Institute of Nuclear Research in Swierk near Warsaw followed by arrests and trials of several scientists.

The work of all schools of higher education was immediately suspended, but there was a protest of teachers and students in Wrocław Technical University where several people including the vice-chancellor were badly beaten. We know about strikes in the Warsaw Agricultural Academy, the Mining Academy in Krakow, and the University of Lublin. The senate of the oldest Polish University, the Jagiellonian University in Kraków, sent a letter of protest with a demand for the release of the arrested people.

The number of students and staff arrested is not known. In the University of Warsaw more than 40 people were either interned or arrested. An overall estimate based on this figure might be misleading because the University of Warsaw has been under the special care of the authorities for many years.

Young people, those between 18 and 25, seem likely to be the main losers: they will lose if they decide to fight, because there is no chance of winning; they will lose if they decide to give up, because of the devastating effects of conformity. The generation gap between them and us, already felt during their strike, seems to be widening. The students prefer to follow the heroic pattern of the young generation during World War II and do not want to give up the only experience they have gained by their own efforts, the experience of the past year.

As yet almost nothing of importance has been changed in scientific institutions by martial law. True, in all universities, as well as in all schools and in most institutes, there are military commissars who are acting not directly but through the existing administration.

But we are only at the beginning of the new road. It seems almost certain that many teachers at all levels will lose their jobs. In secondary schools and in all institutions of public administration the authorities have

already demanded written declarations of loyalty, and those who refuse to sign are fired. A similar type of verification of staff and students is expected in the universities, and it will lead to the replacement of all elected bodies by nominees. Many scientists will probably lose the opportunity to continue their scientific work, as happened in Czechoslovakia.

The outlook is not optimistic. We may expect the worst members of the scientific community to move to the front of the scene. It is a well established tradition to ignore such people; but in the present situation this should not be followed. Scientists who try to abolish what has been achieved should be named and ostracized by the scientific community all over the world. And those who suffer for the defence of scientific freedom should feel the moral, and perhaps also material, support of all their friends and colleagues, first of all here in Poland, but also abroad. If we fail to condemn the former and to provide effective support for the latter our defeat may become a catastrophe.

Not the baron

SIR — Sir Peter Medawar asks in a book review (*Nature* 28 January, p.351) "was it not Cuvier who named a fossil ichthyosaurus *Homo diluvii testis* . . .?". It was not: the fossil (now on display in the Teyler Museum, Haarlem) was so called by J.J. Scheuchzer (1726)¹. Scheuchzer also describes the fossil in a letter published in *Philosophical Transactions of the Royal Society of London*². Baron Cuvier, visiting the Netherlands as a member of a commission sent by Napoleon, confirmed his own identification of the fossil as a giant salamander by carefully removing some of the matrix to reveal the forelimbs (illustrated in Cuvier, 1824)³.

JULIE HAMILTON

University of Oxford, UK

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US spoken

SIR — The editor's leftist leanings have been quite evident of late, but isn't the sentence (*Nature* 14 January, p.86), "United States users of the telephone will most immediately discover that it costs them more to use the local telephone service as if it were unmetered water" (italics mine), not only lacking in lucidity but also bending a bit too far left when it employs "United States" as a general adjective? We are Americans, by God!, not "United Statesers" — and the adjective is *American!* I and others proud of America deplore your emasculation of our rightful name, especially when it is to indulge the arrogance of leftists in Nicaragua, Mexico, or wherever, who never refer to themselves as "Americans", but at the same time, with a malevolent dog-in-the-manger attitude, wish to deny us that distinctive title.

EDWIN E. ROSENBLUM

New York, America

What about the Canadians? *Editor*